Pathways to Recovery and Loss of Nephrons in Anti-Thy-1 Nephritis

WILHELM KRIZ,* BRUNI HÄHNEL,* HILTRAUD HOSSER,* TAMMO OSTENDORF,† SOEREN GAERTNER,† BETTINA KRÄNZLIN,‡ NORBERT GRETZ,‡ FUJIO SHIMIZU,§ and JÜRGEN FLOEGE †

*Institute of Anatomy and Cell Biology, University of Heidelberg, Germany; †Division of Nephrology and Immunology, Klinikum Aachen, Germany; ‡Medical Research Center, University of Heidelberg, Mannheim, Germany; and §Institute of Nephrology, Niigata University, Japan.

Abstract. The present histopathologic study of anti-Thy-1 models of mesangioproliferative glomerulonephritis in rats provides a structural analysis of damage development and of pathways to recovery and to nephron loss. As long as the disease remains confined to the endocapillary compartment, the damage may be resolved or recover with a mesangial scar. Irreversible lesions with loss of nephrons emerge from extra-capillary processes with crucial involvement of podocytes, leading to tuft adhesions to Bowman's capsule (BC) and subsequent crescent formation. Two mechanisms appeared to be responsible: (1) Epithelial cell proliferation at BC and the urinary orifice and (2) misdirected filtration and filtrate spreading on the outer aspect of the nephron. Both may lead to obstruction of the tubule, disconnection from the glomerulus, and subsequent degeneration of the entire nephron. No evidence emerged to suggest that the kind of focal interstitial proliferation associated with the degeneration of injured nephrons was harmful to a neighboring healthy nephron.

Glomerular diseases starting with mesangiolysis have a high probability of recovery. However, not all nephrons recover; some always undergo destruction; we therefore raised the following questions: (1) what is the crucial stage of damage that determines the subsequent recovery or progression and (2) what are the sequences of events leading either to the restitution or to the degeneration of the respective nephron? Thus, our primary question was not why a nephron degenerated or recovered but how these outcomes were achieved. A glomerulus, and all the more a nephron, are both complex structures; we therefore wanted to analyze to which extent the loss and, on the other hand, the reestablishment of the higher order structure were decisive for damage progression and recovery, respectively.

The most common models used to study mesangiolysis and damage development starting therefrom are those that induce mesangial cell lysis with antibodies against the Thy 1.1 antigen of mesangial cells; the resulting disease is generally referred to as anti-Thy-1 nephritis. The classical experiments were performed either with polyclonal antibodies (1,2) or with the monoclonal antibody OX-7 (3). More recently, the monoclonal antibody 1-22-3 (4) has been shown to produce more severe damage. Administration of these antibodies in rats leads to a brisk complement-dependent lysis of the mesangial cells followed by the development of a mesangioproliferative glomerulonephritis.

We used both the OX-7 and the 1-22-3 antibodies in two originally separate studies. Because disease development was more or less identical in both models, we combined both studies. This permitted us to analyze complete pathways of damage development and to provide step-by-step sequences of events leading from mesangiolysis via various intermediate stages to either the recovery or loss of a nephron. We think that these results are of considerable general interest when asking how nephrons degenerate in chronic renal disease.

Material and Methods

The experimental design of both studies was approved by the local authorities according to the German law for protection of animals.

OX-7 Study

Male Wistar rats (Charles River; body weight, approximately 180 g) were used. Three groups of rats were studied, each comprising of four experimental and two control animals. The rats were fed a standard diet with 19% protein and had access to tap water ad libitum. The disease was induced by intravenous (tail vein) injection of the OX-7 anti-Thy-1 antibody; 1 mg/kg body wt dissolved in 0.9 NaCl was administered; controls received the vehicle only.

Rats from group 1 were sacrificed by whole body perfusion (see below) 2 d after disease induction; rats from group 2, 9 d after; and those from group 3, 30 d after. One day before the sacrifice, 24-h urine collections were performed and used to determine total protein excretion.
To study damage development during the critical period between day 1 and 6 in more detail, supplementary experiments were performed. Additional male Wistar rats (180 g body wt) were treated as the others and sacrificed six at a time (four experimental and two control rats) on days 1, 2, 3, 4, and 6 by total body perfusion like the others.

1-22-3 Study
Male Wistar rats (Janvier, France; approximately 300 g body wt) were used. Three groups of rats were studied, each comprising eight experimental and three control animals. They were fed a standard diet with 19% protein and had access to tap water ad libitum. One week before disease induction, all animals were subjected to uninephrectomy (right kidney) following standard protocols under ketamin/xylacin anesthesia (100 mg/6 mg per kg body weight, intramuscularly). The disease was induced by intravenous (tail vein) injection of 1 mg of the 1-22-3 antibody per animal dissolved in 0.9 NaCl according to Cheng et al. (5); controls received the vehicle only. The rats from group 1 were sacrificed by whole body perfusion as described previously (6).

Animal Perfusion
The animals were fixed by total body perfusion as described previously (7). Briefly, under Nembutal anesthesia (50 mg/kg body weight, intraperitoneally), the abdomen was opened and a cannula was retrogradely inserted into the abdominal aorta below the exit of the renal arteries. Without prior flushing, the animals were directly perfused with fixative at a pressure of 220 mmHg for 3 min. In the OX-7 study, two fixative solutions were used: (1) 2% glutaraldehyde phosphate buffer solution (GA-PBS) supplemented with 0.05% picric acid (pH 7.4; osmolarity, 485 mm osmol/L) and (2) 2% paraformaldehyde PBS (PFA-PBS; pH 7.4; osmolarity 900). In each group, three experimental and one control animal were perfused with the GA-PBS solution; the remaining animals were perfused with the PFA-PBS solution. Also, the supplementary animals were subdivided and processed according to both protocols (two experimental, one control in each subgroup). The GA-PBS–perfused material was preferentially used for the structural and the PFA-PBS–perfused material for the immunocytochemical studies. In the 1-22-3 study, all rats were perfused with PFA-PBS following the same protocol. After perfusion, the kidneys were removed and cut into 1- to 2-mm-thick slices.

Structural Studies
Slices from kidneys of all animals were postfixed in 2% GA-PBS overnight; thereafter, they were washed and cut into small blocks (2 × 3 mm) of cortex. These blocks were postfixed in OsO₄ (1% for 2 h) and subsequently dehydrated and embedded into Epon by standard procedures. Semithin (1 µm thick) sections from four different blocks of each animal and, in addition, series of semithin sections (300 sections) of selected blocks from animals from each group as well as ultrathin sections of selected areas were cut on a Ultracut microtome (Leica, Nussloch, Germany) using a diamond knife. Semithin sections were stained according to Richardson et al. (8) and examined with light microscopy (LM); ultrathin sections were stained with uranyl acetate and lead citrate and studied with transmission electron microscopy (TEM).

Light Microscopy
A thorough qualitative analysis led to the subdivision of the structural changes into five categories, which served as a basis for a quantitative evaluation (damage score). The following categories of changes were distinguished:

1. Glomerular profiles without obvious changes
2. Glomerular profiles with early lesions (mesangiolysis, mesangial balloons both with and without proliferation)
3. Glomerular profiles with changes most likely indicating repair (mesangial scars, adherent scars, neck segments)
4. Glomerular profiles with lesions potentially progressing to more severe damage (aneurysms, all kinds of tuft adhesions to BC with and without cellular or matrix crescents)
5. Lesions indicating loss of the respective nephron (collapsed glomeruli, glomerular cysts)

The various types of lesions were counted in random sections from four blocks of each animal, resulting in the evaluation of a minimum of 80 glomeruli from each animal. To establish a damage score, lesions of category 2 were factored with 1, of category 3 with 2, of category 4 with 3, and of category 5 with 4. The sum was then divided by the total number of glomeruli, including those of category 1. The section series were used to trace a certain lesion to gain insight into its extension and its association with other injuries. The lesions and questions which were evaluated are given in Table 2.

In the OX-7 study, mean glomerular tuft volume was measured with a semiautomatic image analysis system (VIDS IV; AtTektron, Düsseldorf, Germany) as described previously (9). Briefly, for each rat, a minimum of 60 consecutively encountered glomeruli in 1 µm Epon sections were analyzed under direct visualization in a blinded manner; sclerotic glomeruli were excluded from the analysis.

Transmission Electron Microscopy
Analysis on the EM level was performed in a qualitative manner. From areas of interest selected in the semithin sections, ultrathin sections were cut and studied with TEM.

Immunocytochemistry
For immunocytochemistry with the avidin-biotin-technique, PFA perfused tissue was embedded in paraffin and 4 µm sections were prepared. To optimize detection, the sections were microwaved five times for 5 min at 600 W in 0.01 M sodium citrate buffer (pH 6.0) after dewaxing. Aldehydes were blocked with 0.1 M ammonium chloride, nonspecific bindings of avidin and biotin with the Avidin/Biotin Blocking Kit, (Vector-Alexis, Grünberg, Germany), and endogenous peroxidase was blocked with 3% H₂O₂. Afterwards, the sections were stained with the ready-to-use Vectastain Elite ABC Kit Peroxidase, (Vector-Alexis, Grünberg). As substrate-chromogen, we used diaminobenzidine (DAB Kit, Vector-Alexis, Grünberg).

The following antibodies were used:
1. Monoclonal mouse anti-rat synaptopodin antibody (G1; 1:2), kindly provided by Dr. Peter Mundel, Albert-Einstein-College of Medicine, NY
2. Polyclonal rabbit anti podocin antibody (1:2000), kindly provided by Dr. Peter Mundel, Albert-Einstein-College of Medicine, NY
3. Monoclonal mouse anti-rat ED-1 antibody (1:150); Serotec, Biozol Diagnostica, Eching, Germany
4. Monoclonal mouse anti-rat proliferating cell nuclear antigen (PCNA) antibody (1:10.000); Abcam Ltd, Cambridge, GB
5. Monoclonal mouse anti-α-smooth muscle actin (α-SMA) antibody (1:100); Biocarta Europe, Hamburg, Germany
6. Polyclonal rabbit anti-rat type I collagen antibody (1:100); Biotrend, Köln, Germany
Table 1. General data, including the results from linear regression analysis between protein/albumin excretion and damage score

<table>
<thead>
<tr>
<th>OX-7 Study</th>
<th>2 d</th>
<th>9 d</th>
<th>30 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Co</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kidney weight (g) (both kidneys)</strong></td>
<td>2.7/2.2</td>
<td>2.8 ± 0.2</td>
<td>3.1/3.4</td>
</tr>
<tr>
<td><strong>Protein excretion (mg/24 h)</strong></td>
<td>5.7/4.8</td>
<td>29 ± 35.9</td>
<td>8/10.8</td>
</tr>
<tr>
<td><strong>correlation with damage score</strong></td>
<td>r = 0.22</td>
<td>r = 0.86</td>
<td>r = 0.92</td>
</tr>
<tr>
<td><strong>Mean glomerular tuft volume (10⁶ μm³)</strong></td>
<td>1.0 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td><strong>Monocytes/macrophages (n/100 glomeruli)</strong></td>
<td>87/99</td>
<td>478.0 ± 43.8</td>
<td>973 ± 12.1</td>
</tr>
<tr>
<td><strong>α-SMA-positive area (% glomerular area)</strong></td>
<td>1.1/2.0</td>
<td>4.7 ± 3.9</td>
<td>61.2 ± 9.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1-22-3 Study</th>
<th>2 wk</th>
<th>5 wk</th>
<th>10 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Co</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kidney weight (g) (left)</strong></td>
<td>2.6 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td><strong>Day 10 protein/albumin excretion (mg/24 h)</strong></td>
<td>15.4 ± 7.8/0.2 ± 0.1</td>
<td>96.4 ± 37.1/40.1 ± 18.8</td>
<td>9.8 ± 5.1/0.3 ± 0.1</td>
</tr>
<tr>
<td><strong>correlation with damage score</strong></td>
<td>r = 0.40/r = 0.14</td>
<td>r = 0.80/r = 0.82</td>
<td>r = 0.74/r = 0.86</td>
</tr>
<tr>
<td><strong>Day 30 protein/albumin excretion (mg/24 h)</strong></td>
<td>14.2 ± 4.9/0.5 ± 0.3</td>
<td>38.5 ± 38.5/16.0 ± 1.0</td>
<td>14.2 ± 3.2/0.4 ± 0.2</td>
</tr>
<tr>
<td><strong>correlation with damage score</strong></td>
<td>r = 0.71/r = 0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 65</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>protein/albumin excretion (mg/24 h)</strong></td>
<td>9.5 ± 2.6/0.7 ± 0.7</td>
<td>58.7 ± 46.1/26.2 ± 22.1</td>
<td></td>
</tr>
<tr>
<td><strong>correlation with damage score</strong></td>
<td>r = 0.91/r = 0.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Lesion and question evaluation

<table>
<thead>
<tr>
<th></th>
<th>OX-7 Study</th>
<th>1-22-3 Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 days</td>
<td>9 days</td>
</tr>
<tr>
<td></td>
<td>Co (n = 2)</td>
<td>Ex (n = 4)</td>
</tr>
<tr>
<td>Assessment of structural changes in random sections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injured glomeruli (% of total)</td>
<td>4.4 100 2.9 98.6</td>
<td>1.8 86.6</td>
</tr>
<tr>
<td>Early injuries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mesangioysis</td>
<td>0 97.8 0 70.9</td>
<td>0 4.5</td>
</tr>
<tr>
<td>mesangial balloons</td>
<td>0 0 0 39.2</td>
<td>0 4.5</td>
</tr>
<tr>
<td>Changes indicating repair</td>
<td>3.9 0.5 1.2 0.2</td>
<td>0 0 65.0</td>
</tr>
<tr>
<td>mesangial scars</td>
<td>3.9 0.5 1.2 0.2</td>
<td>0 0 65.0</td>
</tr>
<tr>
<td>adherent scars</td>
<td>0 0 0 0</td>
<td>0 0 63.0</td>
</tr>
<tr>
<td>neck segments</td>
<td>0 0 0 0</td>
<td>0 2.4</td>
</tr>
<tr>
<td>Progressive injuries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aneurysms</td>
<td>0 0.5 0 1.2</td>
<td>0 0</td>
</tr>
<tr>
<td>adhesions without crescent</td>
<td>0 0.5 0 2.0</td>
<td>0 0.9</td>
</tr>
<tr>
<td>with cellular crescent</td>
<td>0 0 0 8.8</td>
<td>0 1.2</td>
</tr>
<tr>
<td>with matrix crescent</td>
<td>0 0 0 9.5</td>
<td>0 0.3</td>
</tr>
<tr>
<td>Lost nephrons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(atubular glomerular remnants)</td>
<td>0.5 0.7 1.7 5.9</td>
<td>0.9 14.8</td>
</tr>
<tr>
<td>Assessment of structural changes in serial sections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% at which a mesangial balloon had formed an adhesion to Bowman’s capsule</td>
<td>NT 43 (n = 44)</td>
<td>NT</td>
</tr>
<tr>
<td>% at which a mesangial scar was coupled with an adhesion to Bowman’s capsule</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>% at which a tubular neck segment was combined with an adherent scar</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>% at which collapsed glomerulus or a glomerular cyst were disconnected from the tubule</td>
<td>NT</td>
<td>100 (n = 3)</td>
</tr>
<tr>
<td>% at which an intact glomerular profile belonged to a repaired glomerulus with a residual injury (mesangial scar; adhesion) at another site</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Estimation of the fraction of glomeruli that had developed a tuft adhesion (% of total)</td>
<td>NT</td>
<td>44</td>
</tr>
</tbody>
</table>
7. Polyclonal rabbit anti-human WT-1 antibody (1:100); Santa Cruz Biotechnology, Heidelberg, Germany
8. Polyclonal rabbit anti-human CD2AP antibody (1:3000); Santa Cruz Biotechnology, Heidelberg, Germany

For all antibodies, negative controls were used in which the primary antibody was omitted. All control sections were negative.

In the **OX-7 study**, a quantitative assessment of monocytes/macrophages and of α-SMA positivity was done with methyl Carnoy fixed tissue derived from three additional animals in each group as described previously (10). Primary antibodies were identical to those described previously (10,11). To obtain mean numbers of infiltrating monocytes/macrophages in glomeruli, more than 30 consecutive cross-sections of glomeruli (range, 31 to 100) were evaluated and mean values per kidney were calculated. For the evaluation of the immunoperoxidase stain for α-SMA, each glomerular area was graded semiquantitatively and the mean score per biopsy was calculated as described previously (10).

**Statistical Analyses**

Linear regression analysis (SigmaStat, SPSS Science) was employed to examine the correlation between the damage score and the urinary excretion of albumin and/or protein from each animal.

**Results**

The structural changes in both studies were virtually the same. Thus, the analysis could be based on material from rats that were sacrificed at 1, 2, 3, 4, 6, 9, and 30 d (OX-7 study) and 2, 5, and 10 wk (1-22-3 study) after administration of the respective antibody. With both antibodies, the interanimal variation of changes was high, ranging from rats with very little damage at all up to rats in which more than 30% of nephrons had degenerated. Apart from the early disease stages, very reasonable correlations were found between the damage score on the one hand and albuminuria and/or proteinuria on the other hand (Table 1).

**Overview of Damage Development**

The damage in the acute phase on days 1 and 2 consisted of severe mesangiolysis, associated with capillary expansion uniformly encountered in every glomerulus (Table 2). This resulted in a dramatic enlargement of the tuft with frequent prolapses of the tuft into the urinary orifice (Tables 1 and 3). Thereafter (days 4 to 6), advanced lesions in many glomeruli (mesangial balloons, aneurysms, tuft adhesions to BC) were encountered next to changes indicating repair, i.e., cellular repopulation in others (Tables 2 and 3).

In the intermediate stage of the disease (comprising days 6 to 9 of the OX-7 study and the 2-wk group of the 1-22-3 study), the damage was no longer uniform, but extremely manifold. Stages of recovery were mixed with stages of damage progression. Most prominent were (Tables 2 and 3): (1) stages of mesangiolysis and capillary expansion that had not proceeded to more severe damage, but instead exhibited cellular repopulation; (2) mesangial balloons; (3) glomerular aneurysms (rarely); and (4) tuft adhesions to BC associated with a mesangial balloon (many of them had developed crescents and showed encroachment of the injury onto the tubule); (5) a small number of glomerular profiles (5.9% after 9 d; 15.6% after 2 wk; Table 2) represented atubular glomeruli.

In the late phase (comprising the 30-d group of the OX-7 study and the 5-wk and 10-wk groups of the 1-22-3 study), the variety of lesions and stages of repair had increased further.

**Table 3.** Frequencies of structural changes from supplementary animals (4 in each group) as assessed in serial sections (left column) and random sections (all other columns)\(^a\)

<table>
<thead>
<tr>
<th>Day</th>
<th>Prolaps of Podocytes toward the Urinary Orifice</th>
<th>Podocyte Bridges between Tuft and BC</th>
<th>Mesangial Balloons</th>
<th>Aneurysms</th>
<th>Tuft Adhesions to BC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Determined in Serial Sections</td>
<td>Total</td>
<td>At Urinary Pole</td>
<td>Determined in Random Sections ((n=260<del>to</del>420))</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>71%  ((n=14))</td>
<td>10.4%</td>
<td>7.0%</td>
<td>3.1%</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>53%  ((n=15))</td>
<td>5.5%</td>
<td>6.1%</td>
<td>3.0%</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>25%  ((n=8))</td>
<td>5.0%</td>
<td>6.4%</td>
<td>2.7%</td>
<td>1.7%</td>
</tr>
<tr>
<td>4</td>
<td>45%  ((n=20))</td>
<td>3.0%</td>
<td>9.0%</td>
<td>3.8%</td>
<td>17.2%</td>
</tr>
<tr>
<td>6</td>
<td>14%  ((n=7))</td>
<td>1.2%</td>
<td>3.0%</td>
<td>1.6%</td>
<td>45.7%</td>
</tr>
</tbody>
</table>

\(^a\) Random sections always greatly underestimate the actual frequencies; note the large differences between the number of podocyte prolapses determined in serial and random sections (first two columns from left). \(n\), number of glomeruli; \(n_1\), number of glomerular profiles.
Glomeruli with an obviously delayed start of any repair were seen adjacent to those that had recovered. In between, the following lesions were prominent (Table 2): (1) mesangial balloons in different stages of repair, most of them associated with adhesions to BC; (2) different kinds and stages of crescent formation with and without encroachment onto the tubule; (3) repaired injuries with different residual changes (mesangial scars, adherent scars, tubular neck segments); (4) atubular glomerular remnants (collapsed glomeruli, glomerular cysts) associated with areas of tubulointerstitial injury in different stages of progression.

In summary, these observations show (1) that regeneration was the dominant process, mostly leading to outcomes with residual changes; (2) that a substantial proportion of nephrons underwent degeneration; and (3) that in about 4% a neck segment had developed. Figure 1 together with Figure 10 summarize the results.

**Early Lesions**

As known from the classic experiments (1–3), the uniform early lesion of this disease was mesangiolysis (Figure 2a). Though the capillaries were extensively expanded, their branching pattern in conjunction with the folding pattern of the GBM was largely preserved, associated with the maintenance of GBM niches (Figure 2, a and b). Mesangial areas had also expanded (albeit apparently less than the capillaries; Figure 2a), contained only few cells, and were almost devoid of any structured matrix. Most cells in mesangial areas were macrophages (Table 1; ED-1 immunostaining not shown). The expansion of capillaries and mesangial areas resulted in total enlargement of the tuft by 78% on day 2 (Table 1). At the urinary pole, capillary loops approached the urinary orifice, resulting in a prolapse of the corresponding podocytes into the tubular lumen (Figure 2, b-d, f). When followed in serial sections, a prolapse of podocytes was encountered in more than 50% of glomeruli in early stages of the disease (Table 3).

Podocytes overlying expanded capillary loops appeared to be stretched, but the overall architecture was widely preserved. Podocytes prolapsing into the urinary orifice showed more severe changes. First, their cell bodies were stretched, suggestive of physical forces (shear stress), dragging them into the tubular lumen (Figure 2, b-d); in individual cases, such podocytes detached and were found within the lumen of the proximal tubule (not shown). Second, these podocytes (in addition to the usual changes such as foot process effacement and accumulation of absorption droplets) frequently exhibited microvillous transformation of the cell surface (Figure 2, b and f), and, most importantly, they established contacts with BC (Figure 2, d-h).

As seen with TEM, these contacts were created by podocyte processes that had pierced through the parietal epithelium and fixed to the peripheral basement membrane (PBM) (Figure 2, f and g). In addition to structural characteristics (foot processes at the GBM; Figure 2g), these cells were identified as podocytes by immunostaining with antibodies to synaptopodin (Figure 2e) and other podocyte-specific proteins (WT-1, podocin, CD2AP; not shown). Most of the bridges were found at the urinary orifice. Around such bridging podocytes, further cell bridges (made up of podocytes and likely of parietal epithelial cells; Figure 2, g and h) between the GBM and the PBM became established and were reinforced by the deposition of extracellular matrix in between them. This frequently led to a close proximity of a perfused glomerular capillary to the initial site of affixation of the tuft to BC (Figure 2f).

**Advanced Endocapillary Damage**

The initial injury of mesangiolysis with capillary expansion frequently advanced to a mesangial balloon and less frequently to a glomerular aneurysm. A fully developed mesangial balloon (Figures 3c and 4c) comprised an entire glomerular lobule. The folding pattern of the GBM was more or less totally lost, leading to large globular structures that were smoothly encircled by the GBM, followed by a layer of attenuated podocytes; microvillous surface transformation and areas with foot process effacement were frequent in such podocytes (Figure 3, c, c1, and e). In the

---

**Figure 1.** Diagrammatic summary of damage developments to recovery and nephron loss.

---

Copyright © American Society of Nephrology. Unauthorized reproduction of this article is prohibited.
case that individual infoldings of the GBM were maintained, they contained properly differentiated podocyte processes (Figure 3b). Inside the balloons, generally very few capillaries were encountered, most of them without any association to the GBM. Otherwise, these balloons were filled with a fluid-rich tissue containing different amounts of star-shaped cells (compare Figures 3c and 4c); collagen fibers were sparse. Between glomeruli with a largely preserved folding pattern of the GBM and those with mesangial balloons, a continuous spectrum of intermediates was encountered (Figures 3a and 4a).

Glomerular aneurysms were comparably rarely found and only in early stages, never beyond day 9 (Tables 2 and 3). They
were blood-filled lobular cysts with merging of mesangial and capillary spaces (Figure 3d). Their walls consisted of the expanded GBM (like that of a mesangial balloon) but were supplemented from the inside by cellular layers of collapsed and compressed capillaries (Figure 3d1). Proliferation was generally not seen inside such structures.

Changes Indicating Repair of Endocapillary Lesions

In lobules in which mesangiolysis had not advanced to more serious damage, cellular repopulation of the mesangium was generally seen. Evident already on day 2 was an increase in the α-SMA positive area, which dramatically augmented thereafter (Table 1). The newly formed mesangial cells were star-shaped with many cell processes (Figure 3a, 4a, and 4b). They were embedded in a fluid-rich matrix that ubiquitously contained loosely arranged type I collagen fibers. Processes of the cells made contacts either with the GBM (Figure 4b) or with collagen fibers (Figure 4b); intercellular junctions were rarely encountered. The podocytes covering the outside of such lobules displayed a fairly normal morphology (Figure 4a). Podocyte processes filling the niches of GBM infoldings were frequently made prominent by a dense assembly of microfilaments (Figure 3b). Glomerular lobules suggestive of advanced repair exhibited a normal branching pattern of capillaries but prom-
Figure 4. Recovery, repair. (a) An “almost” mesangial balloon. The density of capillaries is greatly reduced; however, some infoldings of the GBM with abutment of capillaries are preserved. In contrast to complete mesangial balloons and aneurysms, cellular repopulation of the expanded mesangial spaces is seen throughout. (b) Part of an expanded mesangial area showing star-shaped cells (asterisk) with processes adhering to the GBM (arrow in the enlargement b1) and to collagen fibers (arrowheads in the enlargement b2). (c through f) Stages of repair of a mesangial balloon. (c) Mesangial balloon with very few capillaries and a sparse network of star-shaped cells. (d) Balloon with a peripheral arrangement of capillaries, a sparse network of cells and without infoldings of the GBM. (e) Balloon with peripherally arranged capillaries adjacent to infoldings of the GBM and accompanied by star-shaped cells; clearly less cells are seen in the center of the balloon. (f) Balloon with peripherally arranged capillaries associated with deep (at least partially) infoldings of the GBM (arrows) and a mesangial center that is filled with solidified mesangial tissue. (g) Glomerular lobule with sclerosis of the mesangium, i.e., scar tissue filling the mesangium; shown in (h) at higher magnification. Note the dense assembly of collagen type 1 fibers. a, b, g, and h, OX-7; a and b, day 9; g and h, day 30; c, d, e, and f, 1-22-3, 10 wk. TEM. Bar length: 20 μm in a, c, d, e, and f; 5 μm in b; 2 μm in b1; 0.5 μm in b2 and h; 10 μm in g.
inent mesangial axes that contained a dense, fibrous (type I collagen) matrix, and apparently more cells than usual (Figure 4, g and h).

The repair of a mesangial balloon was different, started later, and even then at varying points in time (even in the 10-wk group, mesangial balloons with no signs of a beginning repair were encountered). The repair appeared to occur in several discernable stages, starting from mesangial balloons with little cell proliferation. Such balloons were large globular structures, smoothly surrounded by the GBM followed by a continuous layer of attenuated podocytes (Figure 4c). They contained a sparse network of branched cells ($\alpha$-SMA positive; not shown) with a gradient in cell density, decreasing from the connection with the glomerular stalk toward the periphery (Figure 4c). The few capillaries were randomly scattered with few of them at a peripheral location adjacent to the GBM. Mesangial balloons in apparently intermediate stages of recovery showed a peripheral rearrangement of capillaries just beneath the expanded GBM (Figure 4d). Suggestively subsequent stages were characterized by peripheral capillaries associated with shallow infoldings of the GBM and accumulation of cells (Figure 4e; $\alpha$-SMA positive, not shown). Most advanced stages of repair contained a dense center of cells and matrix (collagen type I) peripherally trimmed with normal appearing capillaries (Figure 4f).

**Advanced Extracapillary Damage**

In an estimated almost 60% of glomeruli in both studies (Table 2), the disease encroached upon the extracapillary compartment, starting there with shape changes of podocytes, including the formation of podocyte bridges and subsequent tuft adhesions to BC (described above). Many adhesions developed crescents that were the starting point of progression to more severe damage.

Two types of early crescents were distinguished (Figures 5 and 6). First, a cellular crescent characterized by epithelial cell proliferation (Figures 5a and 6a); second, a matrix crescent (Figures 5b and 6b) that showed little cell proliferation but was predominantly filled with a proteinaceous material that appeared to spread in all directions. However, most cellular crescents also exhibited some matrix in between the cells and, vice versa, the matrix crescents contained some cells. Damage progression was different depending on whether cell proliferation or matrix spreading appeared as the leading process; we therefore subdivided the crescents into those with a dominance of cell proliferation and those with a dominance of matrix spreading.

In the case that no major crescent developed, the adhesion appeared to undergo repair that terminated in an adherent scar. Injuries suggestive of intermediate stages of this process consisted of an increase of cells and matrix (collagen type I) within the balloon as well as in the adjacent interstitium. A layer of amorphous (hyalin) matrix without any capillaries was frequently seen immediately beneath the adherent portion of the GBM. In late stages, a collagenous scar (frequently appearing as a fibrocellular or fibrous crescent) interconnected the tuft to the interstitium (Figure 5c).

**Advanced Lesions Suggestive to Develop from a Cellular Crescent**

An adhesion with a cellular crescent may be described as follows (Figures 5a, 6a, and 7a). It consisted of a mesangial balloon, a bridging portion (the actual crescent), and a cap of proliferating interstitial tissue. The mesangial balloon was found in different stages of damage development (Figures 5a and 6a), containing variable amounts of cells and matrix but generally only few capillaries. At the interface to the crescent,
the GBM was attenuated, and local gaps were encountered in advanced stages.

The crescent itself, i.e., the part of the injury between the GBM and the PBM, was frequently quite voluminous, consisting of cells embedded into variable amounts of matrix (Figures 5a and 6a). The cells were heterogeneous; most of them were epithelial in character; they were negative for synaptopodin (Figure 5d) and other podocyte markers (not shown) as well as negative for α-SMA (Figure 5e); a variable amount were positive for PCNA (indicating proliferation; Figure 5c) or for ED-1 (thus were monocytes/macrophages; Figure 5f). The cells covering the flanks of the adhesion were either parietal cells or podocytes (negative or positive for podocyte markers, respectively; Figure 5d). The extracellular matrix was also heterogeneous, consisting of a fuzzy component (basement membrane-like material) and of an amorphous proteinaceous component, like that of a matrix crescent (see below).

The interface between the crescent and the interstitium was generally delineated by the former PBM (Figures 6a and 7a). The normal PBM of the rat is a multilayered basement membrane (12). The basement membrane of the crescent facing the interstitium had obviously developed from the outer layers of the original PBM. The inner layers became dispersed in the center of the lesion, whereas their lateral remnants frequently deviated from the original course and extended toward the GBM, reaching and even fusing with the GBM. Thereby, these portions of the PBM established the scaffold for the cells that formed the circumferential cover of the adhesion toward the urinary space.

Outside the peripheral basement membrane, interstitial cells proliferated in a circumscribed area, leading to a cellular cap consisting of fibroblasts, myofibroblasts, and monocytes/macrophages (Figures 6, a, e, and f). In advanced stages with scar formation, it became difficult to recognize a clear demarcation between the crescent and the interstitium.

Damage progression of a cellular crescent obviously occurred through continuous cell proliferation inside and outside the former PBM (Figure 6c), which enlarged the crescent in all directions. Most adhesions had developed near the urinary pole; the initial portion of the proximal tubule was therefore incorporated into the expanding crescent (Figure 7a). Tracing such glomeruli in serial sections (Table 2; Figure 7b) revealed that the initial tubular portion had collapsed and transformed into solid cords of cells enclosed in a wide envelope of the former tubular basement membrane (TBM). Close to the glomerulotubular junction, cell proliferation was seen inside (epithelial-like cells) and outside (interstitial cells) this PBM/TBM envelope; macrophages were found on both sides. At some distance from the glomerulotubular junction, cell proliferation was encountered only outside the TBM; inside, the tubular cells were found in various stages of decomposition. This process apparently led to the disconnection of the tubule from its glomerulus, leaving behind atubular glomeruli and agglomerular tubules (see below).

Advanced Lesions Suggestive to Develop from a Matrix Crescent

An adhesion with a matrix crescent may also be subdivided into three portions: the mesangial balloon, the crescent, and the interstitial cap (Figures 5b and 6b). Generally, the crescent was considerably larger than the corresponding mesangial balloon. A middle portion of the crescent adhered to the balloon of the tuft; the interface was established by the GBM. The lateral portions of the crescent were separated from Bowman’s space by a parietal epithelium with a normal appearance.

The proteinaceous material that filled a matrix crescent (Figures 5b, 6b, 8a, 8c) had a homogeneous appearance in the Epon-sections and a characteristic opacity in TEM — both features quite reminiscent of the matrix that filled the paraglomerular spaces in models of “classic” FSGS (13,14). Moreover, this matrix consistently stained with the anti–IgG-antibodies that were used as secondary antibodies, suggesting that it contained plasma derived immunoglobulins (Figure 6, d and e). At variance to what is generally encountered in degenerative models of FSGS, this matrix frequently exhibited darker staining areas (Figure 6b) that contained fibrin, clearly identifiable with TEM by the banded pattern of the fibrils (not shown; [15]). In favorable specimens, we observed that such fibrin deposits occurred in cases with defects in the GBM (Figure 6b). A matrix-type crescent also generally contained some scattered cells.

The separation of a matrix crescent from the interstitium was, like a cellular crescent, affected by a BM derived from the former PBM followed, in contrast to a cellular crescent, by a continuous layer of fibroblasts/fibroblast processes (Figure 6b). Outside this layer, a cap-shaped area of a cell-rich interstitial tissue was generally seen.

Damage in the matrix crescent appeared to progress due to ongoing matrix spreading (Figure 8). In the present models, adhesions/crescents had predominantly developed close to the glomerulotubular junction; therefore, a spreading of the matrix immediately encroached onto the outer aspect of the tubule (Figure 8, a-c). From there, the matrix extended further downstream into the space between the tubular epithelium and the TBM. Outside the TBM, a layer of fibroblasts processes was regularly encountered that established a continuous cellular border toward the peritubular interstitium (Figure 8c).

In cases in which this process appeared to be extensive, producing massively expanded paraglomerular and peritubular matrix-filled spaces, destructive changes in the epithelium of the initial tubular segment along with obstruction of its lumen were seen (Figure 8, a and b). This led to a separation of the glomerulus and tubule from one another, followed by a more or less concurrent degeneration of both (see below).

However, this process apparently took a less severe course in most cases. As derived from an obvious sequence of stages, the initial portion of the proximal tubule atrophied but did not degenerate and instead survived as a flat epithelium. Lesions indicative of the first step in this development exhibited a further but not excessive downstream spreading of the matrix on the outer aspect of the tubule (Figure 8c). From there, the matrix penetrated into the intercellular spaces of the epithe-
The next step in damage progression was mesangial balloon-
Figure 6. Crescents — details. (a) Adhesion associated with a cellular crescent. The lesion consists of three portions: (i) an endocapillary portion (highlighted in yellow); (ii) the bridging portion (uncoloured) separated from the former by the GBM; (iii) a cap of proliferating interstitial tissue (together with the tubules highlighted in yellow), separated from the bridging portion by the former PBM (or a derivate thereof). Note that cells at the flanks of the adhesion contain abundant absorption droplets (asterisk); they are most likely podocytes. (b) Adhesion associated with a predominantly matrix-filled crescent. Again, three portions are distinguishable: (i) an endocapillary portion (highlighted in yellow); (ii) a bridging portion (uncoloured); (iii) outside the PBM, the proliferative response of the interstitium (tubules and interstitium are highlighted in yellow). The adherent portion of the GBM has a gap (arrow; verified at higher magnification, not shown). The crescent contains few cells, suggestively including remnant cells of the parietal epithelium (arrowhead) and macrophages (double arrowhead). The widely homogenous
ing (1). **Mesangial balloons** developed slowly within the first four days with the gradual loss of the folding pattern of the GBM, changing a glomerular lobule finally into a ballooned sac of GBM opening toward the glomerular stalk. This process was associated with the loss of most capillaries. Compared with the dense vascularity of an intact glomerular lobule, a fully developed mesangial balloon contained just a few scattered capillaries. How did the capillaries disappear? In conjunction with the gradual loss of GBM folds, the capillaries lost their abutments to the GBM and obviously underwent a kind of controlled decomposition by — as known from previous studies (21,22) — endothelial cell apoptosis, which led to a dramatic reduction in capillary length but preserved the vascular lining. From the opposite process, the sprouting of new capillaries (see below), it may be supposed that the loss of the narrow association to podocytes was relevant.

The most severe early lesion was a **lobular aneurysm**, in which — in contrast to a mesangial balloon — capillary and mesangial spaces merged. Glomerular aneurysms are characteristic of the Habu-venom–induced glomerular disease, where they develop quickly (within a few hours) (23). At variance with a mesangial balloon, the capillaries rupture, leading, in conjunction with the loss of the GBM folding pattern, to blood-filled lobular aneurysms. It appears that as a consequence of the abnormal blood fluxes, ruptured capillaries are simply pushed aside and compressed to the GBM, resulting in the formation of quite irregular, frequently multilayered walls of the aneurysm (23). Aneurysms were only encountered in early stages of the disease; because they were comparably rare, it was impossible to study their further development.

**Mechanisms Leading to Recovery or Repair**

The lesions recovered as long as the disease remained confined to the endocapillary compartment. Repair was fundamentally different when it started from an injury with a more or less preserved folding pattern of the GBM compared with the repair of a mesangial balloon.

In “preballoon” lesions, the capillaries — even if expanded and simplified in their tortuosity — generally had maintained a peripheral position with abutment to the GBM. This obviously allowed an immediate refilling of the expanded mesangial spaces with cells (3,1). As shown previously (24,25), cells from the glomerular stalk migrated into the glomerular tuft and proliferated. These α-SMA–positive cells (26) established fixations to the GBM at sites just beside a capillary. Centrally, these cells were anchored within the newly established network of mesangial cells and matrix (interstitial collagens) that, as a whole, interconnected peripheral portions of the GBM either with the vascular pole or with opposing portions of GBM. Thus, possibly similar to wound closure (27,28), centripetal contractions of the newly formed mesangial cells (acting as chains of cells interconnected by matrix) may have become effective in drawing opposing parts of the GBM closer together. This led to the reestablishment of GBM infoldings and, consequently, to the reestablishment of filtration surface. Within the center of such a lobule, the mesangial tissue changed into a dense scar tissue. Without a doubt, this newly formed tissue — representing the supporting element of the recovered lobule — occupied a larger volume than the mesangium of the virgin lobule.

The repair of a **mesangial balloon** represents a considerably greater challenge. In conjunction with the total loss of the folding pattern of the GBM, most of the capillaries were lost. The repair of a mesangial balloon requires the outgrowth of new glomerular capillaries in coordination with the reestablishment of GBM infoldings. This process mimics to some extent the development of the glomerular tuft during ontogeny; however, there are differences. During ontogeny, the mesangial spaces into which capillaries grow emerge in conjunction with the growth. During repair, expanded mesangial spaces are preexistent and have to be partitioned for individual capillary loops. Nevertheless, the regulatory system may be expected to be the same. Among the players in this process, VEGF-stimulated capillary morphogenesis appears to take a leading role (22,21,25,29,30,31). The present study supports this idea. When comparing mesangial balloons in different stages of repair, the following sequence of events toward repair may be suggested. At the beginning of the regenerative process, a mesangial balloon contained only few capillaries and few star-shaped cells. Mesangial balloons in this stage were found in all groups of animals, even 10 wk after disease induction. Thus, mesangial balloons apparently tended to persist in this stage. Any further progress toward recovery seemed to depend on the assembly of new capillaries at peripheral sites of a balloon; stages with peripheral capillaries but without a concurrent accumulation of mesangial cells were regularly found. There is evidence that signals from podocytes (possibly VEGF [29,32,33]) had attracted the sprouting of capillaries to sites beneath the GBM, because at the sites of a mesangial balloon which had formed an adhesion to BC and at which the GBM was devoid of podocytes, capillaries never assembled. Subsequently, the new capillaries appeared either to attract further mesangial cells to the periphery of the balloon and/or to stimulate multiplication of those that were already present, thereby matrix is of intermediate electron density and contains striped areas of a clearly more dense matrix (double arrows) that represents fibrin (as seen at higher magnification by its banded pattern [1], not shown). On the outside of the PBM, a dense layer of fibroblast processes is seen (small arrows). (c) Immunostaining of PCNA, showing cell proliferation in a cellular crescent. (d, e, and f). Composition of cellular crescents demonstrated by immunostaining of synaptopodin, which identifies the cells at the flanks of the adhesion as podocytes (d), of α-SMA which identifies myofibroblasts within the tuft and outside the PBM in the interstitium (e), and of ED-1 which shows that — in addition to cells in the tuft and the interstitium — a variable amount of cells within the crescent are monocytes/macrophages (f). Many of the cells of the crescent are negative for all three markers, appear epithelial in character (arrows in d, e, and f) suggestively derived from parietal epithelial cells.
increasing the density of mesangial cells surrounding the capillaries. The cells established contacts to the GBM just beside a capillary. As discussed above, centripetal pull of the GBM may have led to new infoldings of the GBM, resulting in a peripheral segmentation of the mesangial balloon with separation of individual capillary loops. In the center of the former balloon, the mesangial tissue became solidified.

This process is remarkable; it ensures the reestablishment of a glomerular lobule with a proper higher order structure, i.e., with peripherally arranged capillaries supported by centrally located mesangial tissue. In addition, the temporal and spatial sequence of events appears to prevent excessive mesangial cell proliferation and matrix deposition.

A major proportion of mesangial balloons were associated with an adhesion to BC (Table 1). Adhesions that did not develop crescents or developed just small crescents generally
entered a repair process. This process consisted of two parts. First, the repair of the endocapillary damage of the balloon appeared to occur as just described. Second, the repair process of the extracapillary damage of the adhesion consisted of the formation of a bridge of scar tissue that developed from both sides, from the mesangium and the interstitium, finally inter-
Figure 9. Final nephron degeneration/tubulointerstitial injury. Panels a, c, and e show areas of final nephron degeneration located beneath the kidney surface (upperside), characteristically leading to a depression; panels b, d, and f show midcortical areas. Two types of glomerular remnants were found, either with collapse (a and b) or with cystic expansion (c and d) of BC; when tracing in serial sections, both types were separated from their tubules. (a) Atubular collapsed glomerulus with perfused tuft remnant and preserved postglomerular perfusion; note the patent efferent arterial (arrow). The area between the glomerulus and the surface contains the formerly corresponding tubules, all in advanced stages of decomposition (arrows). Note the adjacent healthy tubules (asterisks) derived from unaffected nephrons. (b) A very small collapsed atubular glomerulus that extended for only 70 sections (70 μm) in a series. The corresponding tubules have almost completely disappeared; scarce remnants (arrow) are embedded into loose interstitial tissue. (c) Atubular glomerular remnants with expansion of BC. The corresponding scarce tubular remnants (arrows) are intimately intermingled with healthy tubules (asterisk) from unaffected nephrons. Note the adhesion of the tuft remnant (arrowhead). (d) Expanded atubular glomerulus; the tuft remnant contains normal-looking capillaries around
connecting the mesangium with the interstitium. Epithelial cells completely disappeared from the interface between the former mesangial balloon and the interstitium. In the end, the lesion manifested as segmental glomerulosclerosis, frequently associated with a fibrocellular crescent. From previous studies (34,35,36) and from hints in the present study (in the 10-wk group of rats, 20.2% of glomerular profiles were classified as intact, i.e., exhibiting slim mesangial axes Table 2), we know that scar tissue within the mesangium and a crescent may in the long run be subject to a remodeling process, leading to a decrease in its volume. However, we were unable to find a decrease in the ratio of glomeruli with tuft adhesion between the middle and last groups of animals in either of the studies (Table 2). Thus, we found no evidence that an adhesion of the tuft to BC may disconnect, reestablishing a freely floating tuft.

Encroachment of the Disease onto the Extracapillary Compartment — Formation of Crescents

Already very early in the disease, podocytes developed contacts to BC in a great number of cases (Table 3). The mechanism appears to be identical to what has previously been shown in models of crescentic glomerulonephritis in mice (37) and rats (38). Podocytes formed apical projections that penetrated through intercellular clefts of the parietal epithelium and fixed to the PBM. In the previous studies, microvillus transformation of the podocyte surface was the uniform change in podocyte phenotype (migratory phenotype), which preceded the formation of cell bridges. Identical changes were seen in many podocytes in the present study as well.

As in the mouse, the affixation of a podocyte at the PBM appeared to weaken both the adherence of adjacent parietal epithelial cells to the PBM as well as that of the involved podocytes to the GBM, facilitating the fixation of further podocytes to the PBM and, vice versa, fixation of further parietal epithelial cells to the GBM. Such clustered cell bridges enclosed a space that generally filled with an extracellular matrix. Thus the adhesion finally consisted of cells and matrix interconnecting the GBM and the PBM.

The processes accounting for the further progression of damage occurred at the capsular side, outside the parietal epithelium, precisely within a newly created space between the parietal epithelium and its basement membrane. Two processes appeared to produce and to expand this space: epithelial cell proliferation and misdirected filtration toward the interstitium. This led to two types of crescents, either predominantly cellular or predominantly matrix crescents. We have no clues for understanding why in one case cell proliferation dominated while in another misdirected filtration.

With regard to a cellular crescent, previous work suggested that the interposition of podocytes in between parietal cells led to a loss in cell polarity and contact inhibition of parietal cells followed by cell proliferation (37). This may have occurred in the present study as well, but we cannot preclude that the proliferating epithelial cells (or part of them) actually were podocytes that had lost their specific markers as in collapsing FSGS (39). The newly formed cells established intercellular junctions neither with each other nor with the surrounding cells, but generally stayed apart from each other, separated more or less by an amorphous extracellular matrix. These cells and matrix expanded the space between the parietal epithelium and its basement membrane in all directions. The PBM frequently separated into two portions: an outer layer, which delineated the interface toward the interstitium, and an inner layer, which served as a substratum for the cells that separated the peripheral, non-adherent parts of a crescent from the urinary space. In addition to epithelial cells, crescents consistently contained many macrophages.

Matrix crescents are thought to develop by misdirected filtration. In models of classic FSGS, this process has been shown to occur when capillaries contained in an adherent tuft portion came close to the interface with the interstitium, delivering their filtrate toward the interstitium (13,14). In these studies, matrix-filled paraglomerular spaces were encountered that looked identical to the matrix crescents in the present models. Based on the similarity of the situation (tuft adhesion to BC) and the identical appearance of the matrix in LM and TEM, we concluded that misdirected filtration is a major process underlying the formation of a matrix crescent in the present study. Equally, the interstitium responded with the formation of a dense layer of fibroblasts/fibroblast processes that tightly encapsulated the focus of leakage, preventing the filtrate from entering the interstitium proper. The filtrate was thereby forced to spread onto the outer glomerular surface and — especially in cases with adhesions close to the urinary pole — along the glomerulotubular junction and onto the outer aspect of the tubule (see below).

Damage Progression by Epithelial Cell Proliferation

The pathways to nephron degeneration differed according to whether cell proliferation or misdirected filtration was the
dominant process. In the case that cell proliferation dominated, the encroachment of the proliferative process upon the tubule led to collapse obstruction of the urinary orifice, decomposition of the initial tubular segment, and finally to the separation of glomerulus and tubule. Very recently, identical developments have been found in anti-GBM nephritis in mice (40) as well as in crescentic glomerulonephritis in humans (41). This process is therefore likely to be of great impact in inflammatory glomerular diseases. The underlying mechanisms are unknown. The formation of a cellular crescent is associated with the desintegration of the parietal epithelium (37); a comparable process is likely involved concerning the tubular epithelium. Taking into account the results from recent studies in transgenic mice and humans (42,43,44), a crucial role of macrophages may be suggested. The outcome is definitive; the remnants are atubular glomeruli and agglomerular tubules.

Deprived of a luminal load, the tubules of such nephrons underwent decomposition, removal, and replacement by fibrous tissue in a steadily progressive manner. Initially, the tubular epithelium atrophied in a way previously shown (45,46). Ultimately, the cells underwent cell lysis, and their remnants, including the GBM, were obviously removed by macrophages. These observations support previous studies showing that the survival of tubular epithelia depends on their work load (47,48,49).

The glomeruli of such nephrons either collapsed or developed cystic expansions of BC. The former appeared to undergo a slow process of decomposition. The characteristics — podocyte dedifferentiation with loss of the cytoskeleton, detachment of adjacent capillaries from the GBM, collapse of the denuded GBM — are reminiscent of tuft degeneration in collapsing FSGS (39). The latter appeared to develop in glomeruli with some ongoing filtration and in which BC did not withstand expansion. These forms survived for an unknown period, possibly lifelong. Atubular glomeruli have previously been observed in models of non-inflammatory kidney diseases (9,13,50–52), they have been found in humans with proteinuric diseases (53,54), and they are frequent in kidneys with chronic transplant nephropathy (55).

**Damage Progression by Misdirected Filtration**

In cases in which misdirected filtration was the dominant process, its strength seemed to be crucial. If it was strong and maintained for some time, and it led to the separation of glomerulus and tubule and to a more or less concurrent degeneration of glomerulus and tubule, as just discussed. However, more frequent were the cases in which the process of misdirected filtration appeared to come to a stop after some time, providing the chance for repair. The tubular epithelium of such nephrons underwent atrophy but, surprisingly, did not degenerate. The mechanism was remarkable; the filtrate gradually expanded the intercellular clefts between the basolateral cell processes of the proximal tubule epithelium, leading to a progressive atrophy with total loss of cell processes and apical microvilli. The epithelium survived as a simple flat cellular coat, indistinguishable from the parietal epithelium. The result was a nephron with a tubular neck segment, i.e., with an initial thin tubule that, at a short distance from the tubular orifice, passed over into a proximal tubule with no signs of damage. Thus, at variance to models of classic FSGS (9,14,56), where the filtrate spreading may involve the entire proximal convolution (leading sooner or later to obstruction), peritubular filtrate spreading in the present inflammatory models frequently appeared to come to an early stop, allowing this specific course of repair and resulting in an atrophied tubular segment associated with an adherent scar.

In human pathology, circumscribed tuft adhesions to BC close to the urinary pole are called tip changes (57,58). They have been shown to be preceded by an enlargement of the glomerular tuft and a prolapse of the tuft into the tubule (58,57). Moreover, cases in stages of apparent disease resolution were found to be associated with solidified adhesions, and with a delayed start of the normal tubular epithelium (58), i.e., a neck segment. Thus, the similarities between the human and the experimental lesion are far reaching. In agreement with the findings in humans (57,58), the present observations suggest that the crucial precondition for the development of a tip change is the loss of mesangial support (which may be a circumscribed process), leading to a prolapse of a capillary loop into the urinary orifice. Such an event would be more likely the result of an inflammatory than of a degenerative process.

**Relevance of Tubulointerstitial Processes**

The distribution of degenerating nephrons was strictly focal. In random sections, a degenerating nephron was commonly

Figure 10. Schematics depicting the main stages of damage development. A longitudinal section of a glomerulus with vascular and urinary poles is drawn. Smooth muscle cells and extraglomerular and glomerular mesangial cells are shown in dark green; interstitial cells are shown in light green. Podocytes are shown in dark brown, tubular cells in light brown, and endothelial cells in magenta. The GBM separating the endocapillary and exocapillary compartments is drawn as a thick black layer, the multilayered PBM is hatched, and the TBM is again shown homogeneously black. Macrophages and other blood-borne mononuclear cells are shown in violet. (a) Normal glomerulus; (b) glomerulus with ubiquitous mesangiolysis and capillary expansion; (c) repair that started from mesangiolysis; (d) aneurysm; (e) mesangial balloon; (f–h) repair of a mesangial balloon, (f) stage I with peripheral assembly of capillaries beneath the GBM, (g) stage II with reestablishment of peripheral infoldings by centripetal pull (arrow), (h) stage III with contraction and formation of a globular mesangial scar; (i) mesangial balloon with podocyte bridge to BC; (k–m) development of a matrix crescent and tip change, (k) matrix crescent with beginning peritubular matrix spreading, (l) matrix crescent with progressing tubular atrophy, (m) healed tip change; (n–q) development of a cellular crescent and final nephron degeneration, (n) cellular crescent, (o) cellular crescent with encroachment onto the tubule and tubular obstruction, (p) collapsed atubular glomerulus associated with tubulointerstitial injury, and (q) expanded atubular glomerulus (glomerular cyst) in an advanced stage after complete removal of the corresponding tubules.
seen as a circumscribed tissue area, which, when followed in serial sections, consisted of a severely injured glomerulus with its corresponding tubules in various stages of destruction. In recent work, the idea is widely discussed whether excessive protein leakage through an injured glomerular filter and subsequent reabsorption of these proteins may initiate or at least contribute to the destruction of the tubule (59–62). In the present model, such a mechanism did not seem to play a major role. Tubular degeneration in the present models was exclusively encountered subsequent to tubular obstruction near the glomerulotubular junction; there was no evidence that tubular destruction had already started before obstruction. Afterwards, no filtered protein had access to the tubule.

The degeneration of a tubule was always accompanied by vivid interstitial proliferation, generally confined to the immediate surroundings of the degenerating tubule with no signs of penetration between intact tubules. Moreover, in favorable cases, tubular segments that belonged to healthy glomeruli were found to pass through areas of tubulointerstitial injury, but obviously remained unaffected by the degenerative process in their surroundings. First, these observations speak against the idea that disturbances of the postglomerular circulation have a major influence on the progression of the tubulointerstitial injury. Second, these observations have considerable impact on the current discussion about the relevance of tubulointerstitial changes with respect to progression of the disease. Decline in renal function in chronic renal disease is derived from the progressive loss of viable nephrons. Thus, the decisive question that has to be raised in this context follows: Does interstitial proliferation and matrix deposition, as it focally occurs in association with the degeneration of a nephron, initiate the destruction of adjacent, so-far healthy nephrons, with the degenerative process in these nephrons starting in the tubulointerstitium? The answer from the present models is clearly no. In contrast, the way in which a nephron underwent destruction assured that the degenerative mechanism remained confined to the affected nephron. There was no evidence of a nephron-to-nephron transfer of the disease at the level of the tubulointerstitium. This conclusion is in full agreement with previous studies of the Thy-1 model (63,64), with studies of several degenerative models of nephron loss (9,46,65–67), as well as with studies of human cases (13,68).

**Conclusion**

Glomerular injury starting with mesangiolysis may be resolved, recover with a residual scar, or progress to nephron degeneration. The following pathways were uncovered:

- Loss of mesangial support leads to mesangiolysis associated with capillary expansion but not necessarily to the formation of a mesangial balloon. Sustained support by podocytes may preserve glomerular architecture over a certain time period.
- Mesangial balloons are characterized by a more or less complete loss of the folding pattern of the GBM together with the loss of most capillaries. Mesangial balloons have to be distinguished from glomerular aneurysms, which are characterized by a merging of capillary and mesangial spaces.
- Repair of simple mesangiolysis and of a mesangial balloon are fundamentally different. Whereas repair in the former starts with the immediate cellular repopulation of the mesangial spaces, the formation of new capillaries in the latter is the prerequisite of repopulation with mesangial cells. In both cases, centripetal contractions of α-SMA–positive mesangial cells appear to underlie the reestablishment of the GBM folding pattern. The repair of a mesangial balloon with adhesion to BC results in the formation of a mesangial scar that connects, via a gap in BC, to an interstitial scar. Once established, disconnection of tuft adhesions to BC does not appear to occur.
- Progression of the disease to extracapillary damage starts with expansion of the tuft, leading to prolapse of podocytes into the urinary orifice, establishment of podocyte contacts to BC and finally formation of tuft adhesions.
- Tuft adhesions may develop two types of crescents: the cellular or of the matrix type. In cellular crescents, continuous cell proliferation with overgrowth onto the tubular orifice resulted in the obstruction of the tubular lumen. This led to disconnections of the tuft from the glomerulus, thus to the formation of atubular glomeruli and aglomerular tubules. Matrix crescents appeared to develop due to misdirected filtration and progressed due to paraglomerular and peritubular matrix spreading. In the case that these processes were prominent, they led to tubular obstruction and separation of glomerulus and tubule, much like with a cellular crescent. In the case that these processes came to an early stop, they led to the formation of a solidified tuft adhesion located close to the urinary orifice and associated with a tubular neck segment (tip change).
- Degeneration of aglomerular tubules started with epithelial atrophy, progressed to tubular decomposition, and was accompanied by local interstitial cell proliferation. Two types of atubular glomerular remnants were encountered: the cystic forms appeared stable, while the collapsed forms underwent decomposition and removal, albeit slower than the tubular remnants.

**Acknowledgments**

We gratefully acknowledge the work of Rolf Nonnenmacher, who did the excellent art work, of Ingrid Ertel for photographic work, of Cari Adams for help with English syntax, and of Carla Lyko for secretarial help. We thank our colleagues Hermann-Josef Groene, Ralph Witzgall, and Karlhans Endlich for careful reading of the manuscript. The work was supported by a grant KR 546/19 from the “Deutsche Forschungs gemeinschaft.”

**References**


