From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies

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Abstract

Background. Focal segmental glomerulosclerosis (FSGS) is consistently associated with tubular degeneration and interstitial fibrosis, altogether, accounting for the progressive decline in renal function. The mechanisms which link glomerular injury to tubulointerstitial fibrosis are controversial. The present study describes the step-by-step sequence of histopathological events, i.e. the evolution of the injury from the initial lesion in the glomerulus to total nephron destruction.

Methods. The investigation was performed in male hypertensive Fawn-hooded rats (6-, 9-, and 12-month-old) and 14-month-old Milan normotensive rats. The kidneys were fixed by in vivo perfusion and processed for structural investigation. Autopsy materials from human cases of focal segmental glomerulosclerosis and diabetic nephropathy were also examined.

Results. FSGS as seen in rat models consists of collapsed and hyalinized capillaries and mesangial portions which are included within a synechia between the glomerular tuft and Bowman’s capsule. In addition, a synechia generally contains glomerular capillaries which are perfused and continue to filter with the filtrate being delivered into the interstitium rather than into Bowman’s capsular space. Such filtration creates a paraglomerular space on the outer aspect of the parietal epithelium. This space becomes separated from the interstitium by a dense layer of sheet-like fibroblast processes. Associated with the progression to global sclerosis, this space spreads around the entire circumference of a glomerulus; all ‘sclerotic’ tuft portions are eventually contained in this space. Starting from the urinary pole this process also involves the proximal tubule, initially by expanding the tubular basement membrane (TBM) and later, by separating the TBM from its epithelium, thus creating a peritubular space by misdirected filtrate spreading. Similar to the situation observed at the glomerulus this space becomes separated from the interstitium by a layer of fibroblast processes. The final degeneration of the nephron occurs via two pathways. Pathway I whereby development to global sclerosis is dominant or develops concurrently with tubular degeneration, eventually terminating in global and cylindrical remnants of extracellular matrix surrounded by abundant fibrous tissue. Pathway II where the degeneration of the tubule is ahead of damage progression in the glomerulus leading to atubular glomerular cysts.

Conclusion. The present study suggests that severely injured glomeruli may continue to filter with the filtrate spreading along interstitial routes. Fluid added locally to the interstitium from such ‘extraterritorial’ glomerular capillaries probably is quite different in quantity and composition compared to that from interstitial capillaries. We propose that this kind of abnormal addition of fluid to the interstitium is the essential mechanism accounting for interstitial progression of the disease. Similar histopathological phenomena in human kidneys with focal segmental glomerulosclerosis suggest that the pathogenetic pathways defined in the rat models operate in human disease as well.

Key words: Chronic renal disease; glomerulosclerosis; interstitial fibrosis; misdirected filtration; renal histopathology

Introduction

The progressive loss of renal function in chronic renal disease results from progressing nephron loss. As shown by histology, in most cases of chronic renal failure nephron loss follows a pattern known as focal segmental glomerulosclerosis (FSGS) which is associated with tubular degeneration and interstitial fibrosis. Focal segmental glomerulosclerosis is used here in the
classic definition which includes both primary and secondary forms of FSGS [1]. A more recently described pattern of glomerular degeneration, called 'collapsing' FSGS is distinct from the classic form in several respects [2–4] and does not conform to the pathogenetic mechanisms investigated in the present study. In several experimental rat models [5–9] we have shown that the glomerular damage starts by an adhesion of the glomerular tuft to Bowman’s capsule developing into a synechia, i.e. into segmental glomerulosclerosis. The further progression of the damage to global sclerosis, tubular degeneration and interstitial fibrosis is less well-understood and appears to be less uniform. Several mechanisms linking glomerular injury to tubulo-interstitial fibrosis have been proposed [10–16]; they may be divided into three groups. First, repeating episodes of glomerular injury lead to a sustained and eventually self-perpetuating production of certain growth factors and chemokines (TGF, PDGF, osteopontin, MCP-1) not only by glomerular but also by tubular and interstitial cells followed by the deposition of extracellular matrix, ultimately resulting in renal fibrosis [17–22]. Second, postglomerular events such as post-capillary hypertension or deranged local production of vasoactive hormones (angiotensin II, endothelin and nitric oxide) reduce peritubular capillary plasma flow and cause rari-fication of post-glomemular capillaries, resulting in local hypoxia and tubular atrophy. Again this is accompanied by upregulation of certain growth factors (TGF, osteopontin) promoting interstitial fibrosis [23–27]. Third, protein leakage through injured glomeruli exposes proximal tubules to excessive protein load. In response, these tubules upregulate inflammatory and vasoactive genes such as MCP-1 and endothelin. These mediators, formed in excessive amounts, are secreted towards the basolateral compartment and give rise to an inflammatory reaction resulting in interstitial fibrosis and renal scarring [28–30] followed by tubular degeneration. It is obvious that these mechanisms are not necessarily exclusive to each other, their effects may be additive. The present study suggests a further mechanism which is fundamentally different from the others but which, nevertheless, may act in concert with the others, as well. We present histopathological evidence that misdirected filtration from segmentally injured glomeruli towards the interstitium contributes to progression of damage, ultimately leading to global sclerosis, tubular degeneration and interstitial fibrosis.

Material and methods

The present investigation was performed using the material of a previous experimental study [8] concerning the development of segmental glomerulosclerosis in male hypertensive Fawn-hooded rats (FHH rats; 6-month-old; body weight (BW) around 300 g). Additional material was obtained from 9- and 12-month-old male FHH rats (BW around 400 g) and 14-month-old male Milan normotensive (MN) rats (BW around 500 g). Like FHH rats, MN rats spontaneously develop focal segmental glomerulosclerosis [31,32]. The kidneys were fixed by in vivo perfusion and processed for structural investigations as described previously [8]. Briefly, after anaesthesia with Nembutal (1.2 ml/kg BW) the abdominal cavity was opened and the kidneys were subjected to retrograde perfusion via the abdominal aorta without prior flushing of the vasculature. Perfusion pressure in the perfusing apparatus was set at 220 mmHg; the fixative contained 4.0% formaldehyde in phosphate-buffered saline (PBS; calcium and magnesium free, pH 7.4). After 3 min of perfusion, the left kidney was clamped and removed and processed for structural investigations. With respect to the right kidney, perfusion was continued for another 5 min by a 18% sucrose solution acting as a cryoprotectant for subsequent shock-freezing. These kidneys were used for immunocytochemical studies (to be published elsewhere).

Structural investigations

After removal, the left kidney was cut into slices; a middle piece was processed for light microscopy following standard procedures. After embedding in paraplast, 4 µm thick sections were cut and stained with haematoxylin eosin. For transmission electron microscopy (EM) and high resolution light microscopy, slices of renal cortex were post-fixed in 2% glutaraldehyde–PBS solution overnight, cut into small blocks and post-fixed in 1% OSO4 for 2 h. After dehydration in a graded series of ethanol the samples were embedded in Epon. Semithin sections (1 µm thickness) were cut with glass knives on a Reichert Ultracut, stained with methylene blue and examined by light microscopy (Polysar 2, Reichert). Ultrathin sections were cut with a diamond knife on a Reichert Ultracut and stained with 5% uranyl acetate for 15 min followed with lead citrate for 2 min. They were evaluated in a Philips EM 301. Several series of 1 µm thick sections were prepared from kidney cortices of both FHH and MN rats. Also mixed sections series were prepared which included ultrathin sections (to be studied by EM) taken at variable intervals within a series of semithin sections.

Human tissues

Autopsy materials (paraffin blocks from routine processing) from cases of focal segmental glomerulosclerosis and diabetic nephropathy were obtained from the Departments of Pathology in Heidelberg (Prof. Dr H. F. Otto) and Basel (Prof. Dr M. J. Mihatsch). Sections (4 µm) were stained by the trichrome technique after Goldner, examined and photographed on a Leica Polysar. In addition, the unaffected pole of a surgically removed tumour kidney was received from Prof. Dr S. Peter (Department of Urology, Städtisches Krankenhaus Darmstadt). Tissue blocks were routinely fixed in formaldehyde by immersion, processed by standard procedures, embedded in Paraplast, sectioned and examined as described above. In this tissue widespread FSGS was found.

Results

Experimental models

Segmental glomerulosclerosis in experimental rat models (FHH rat, MN rat and chronic Masugi nephritis) consists of a broad synechia between the glomerular tuft and Bowman’s capsule [8,9,31]. Such segmental synechiae are composed of collapsed and/or hyalinized GBM formations (representing former
capillaries and/or mesangial areas) included within a paraglomerular space. Towards the cortical interstitium this space is walled off by a layer of sheet-like fibroblast processes which has quite obviously developed in association with the formation of the synechia. Towards the urinary space the synechia is separated by the parietal epithelium which adheres to the GBM at the flanks of the synechia; the interface between the collapsed/hyalinized tuft portions (making up the synechia) and the ‘intact’ tuft portions is represented by branches of mesangium which, towards the adhesion, have lost their covering by podocytes. The paraglomerular space is apparently filled by a proteinaceous fluid which, as suggested in a previous communication [8], is derived from misdirected filtration or exudation out of capillaries which are trapped within the adhesion, but are still perfused. This space tends to spread on the outer surface of Bowman’s capsule by separating the parietal epithelium from its basement membrane. Eventually, via the vascular pole this process encroaches upon other glomerular lobules initiating the progression to global sclerosis (Figure 1a).

Fig. 1. Segmental glomerulosclerosis; progression of damage with involvement of the tubule. Accompanying drawings illustrate some details: intact tuft portions are hatched, sclerotic tuft portions are shown in black, parietal and tubular epithelia are densely stippled, paraglomerular and peritubular spaces are loosely stippled, Bowman’s space, tubular lumina and patent capillary lumina within sclerotic tuft portions are shown in white; the fibroblast border to the interstitium is indicated by a hatched line. (a) Glomerular profile with segmental sclerosis extending from the vascular (VP) up to the urinary pole (UP). The ‘sclerotic’ tuft portions consisting of collapsed and/or hyalinized tuft remnants are located outside Bowman’s capsule enclosed in a paraglomerular space. The sclerotic tuft portion contains patent, obviously perfused capillaries (small arrows). The paraglomerular space is separated from Bowman’s space by the parietal epithelium and from the surrounding interstitium by a continuous layer of thin sheet-like fibroblast processes (arrowheads). The paraglomerular space extends across the vascular pole onto the opposite hemisphere of the glomerulus (hatched arrow). At the urinary pole the extension of the paraglomerular space into an expanded TBM is seen (long arrows). (b) Urinary pole of a glomerulus with segmental sclerosis. The sclerotic tuft portions (not really shown in this picture; the final point is just seen in the upper right corner) are contained in a paraglomerular space which extends alongside the urinary pole onto the tubule (hatched arrows); the thickened TBM (long arrows) represents a peritubular space. Both, paraglomerular and peritubular spaces are delineated from the cortical interstitium by a continuous layer of thin sheet-like fibroblast processes (arrowheads). TEMs: (a) from MN rat at 14-month-old, ×~400; (b) from FHH rat at 6-month-old, ×~800.
In the present study we show that this space may even extend across the urinary pole onto the proximal tubule. First, by expanding the tubular basement membrane (TBM; Figure 1b), and later, by separating the TBM from its epithelium (Figure 2a,b) a peritubular space is created which is an equivalent of the paraglomerular space. This space fully envelopes the initial portions of the proximal tubule, and like the paraglomerular space, is separated from the cortical interstitium by a layer of fibroblast processes, which has obviously been established in response to the formation of a peritubular space (Figures 1–4). This space progressively extends along subsequent portions of the proximal tubule.

The structural lesions developing subsequent to this stage, may be grouped in two different patterns reflecting different pathways of nephron degeneration. A first pathway in which progression to global sclerosis is dominant or in which global sclerosis and tubular degeneration develop concurrently and, a second pathway in which tubular degeneration is ahead of the degenerative process in the corresponding glomerulus.

**Pathway I: development to global sclerosis is dominant or develops concurrently with tubular degeneration**

As described in detail elsewhere [8,9], the decisive step in the progression from segmental to global sclerosis is the progressive expansion of the paraglomerular space up to the vascular pole. This allows the sclerotic process to spread to all other lobules; eventually the entire glomerulus is affected. As a consequence, a urinary space disappears; most former capillaries and mesangial areas are collapsed and/or hyalinized and have been enclosed in a giant paraglomerular space (Figure 2a). By tracing the patent capillaries through such ‘glomeruli’ in a series of 1·μm sections, the continuity of a blood flow route from the afferent to the efferent arteriole could be verified in several cases. Thus, these capillaries which have come to lie outside of Bowman’s space are perfused. They represent, so to speak, ‘extraterritorial’ glomerular capillaries. It is plausible to assume that they continue to produce some filtrate and to deliver it into the paraglomerular space.

The final degeneration of such an injured glomerulus is always associated with the degeneration of the corresponding tubule (Figure 3). As described above, the paraglomerular space extends onto the proximal tubule. In the vicinity of glomerular profiles in this stage of sclerosis development, many tubular profiles are generally found which are enclosed by a peritubular space. The continuity of the paraglomerular spaces across the urinary pole into the peritubular space has been traced and verified in many cases. First, the TBM at the urinary orifice surrounding the initial portions of the proximal tubule expands, i.e. increases in thickness (Figure 1b). Later, as clearly seen in Figures 2a,b, the proximal tubule epithelium fully disconnects from its basement membrane. Thereby, large fluid-filled peritubular spaces are formed. This sequence of events, i.e. expansion of the TBM followed by disconnection from its epithelium, is seen in progressively more distal portions of the proximal tubule. It appears that the entire convoluted part of the proximal tubule may finally be affected, at least with respect to expansion of the TBM in width. The various stages of progression of the injury in the tubule are depicted in Figure 4. First, a patent proximal tubule (intact epithelium) is surrounded by an expanded TBM completely enclosed by a sheath of thin sheet-like fibroblast processes (Figure 4a). With progression of the injury the epithelium flattens and gradually loses its characteristics; at this stage the tubular lumen is frequently dilated (Figure 4b). Later, the tubule collapses, the epithelium transforms into solid cellular cords without any lumen. The surrounding matrix cylinders (the former TBM) are transformed into a heavily wrinkled envelope (Figure 4c). These structures usually become separated from the interstitium by interposition of a cover of thin sheet-like fibroblast processes. The final stages of tubular degeneration are characterized by gradual disappearance of any remnant epithelial cells. The remaining ‘tubular’ profiles consist of heavily wrinkled matrix mantles which enclose more or less empty, pale staining, irregular spaces (Figure 4d). In between these degenerating ‘tubules’ abundant loose interstitial tissue is encountered (Figure 4b–d), ultimately fibroblasts are seen penetrating the ‘tubular’ matrix remnants.

The degeneration of the glomerulus advances along with the degeneration of the tubule (Figure 5). When one compares Figure 2 with 6 and Figure 3 with 5 a major difference is immediately apparent, i.e. the absence of paraglomerular spaces: they have disappeared, the glomeruli have collapsed. Patent vessel routes through such glomerular profiles could no longer be traced. Moreover, the barrier of fibroblast processes between the sclerotic glomerular remnant and the interstitium dissociates locally. Fibroblasts are seen crossing the border approaching the collapsed tuft structures. The result is a ball of extracellular matrix with few remaining cells surrounded by fibrous tissue (Figure 6).

**Pathway II: degeneration of the tubule is ahead of the development to global sclerosis**

This pathway is less frequent. Here the spreading of the paraglomerular spaces across the urinary pole onto the corresponding tubule apparently proceeds more rapidly than progression of segmental to global sclerosis. In heavily damaged areas with collapsed obstructed tubules all of which are surrounded by peritubular matrix and enclosed by fibrous tissue, quite frequently, glomerular cysts containing small tuft remnants are seen (Figures 7b, 8). We conclude that such dilated glomerular profiles reflect a situation where progression of damage at the glomerular level (from segmental to global sclerosis) is delayed compared to progression of tubular degeneration. Such glomerular cysts appear to develop from early tubular obstruction when a misdirected filtrate...
Fig. 2. Advanced glomerulosclerosis with extension of the process to involve the tubule. Accompanying drawings illustrate some details: intact tuft portions are hatched, sclerotic tuft portions are shown in black, parietal and tubular epithelia are densely stippled, paraglomerular and peritubular spaces are loosely stippled, Bowman's space, tubular lumina and patent capillary lumina within sclerotic tuft portions are shown in white; the fibroblast border to the interstitium is indicated by a hatched line. (a) Glomerular profile which is totally 'sclerotic': the sclerotic tuft portions are enclosed in a tremendous paraglomerular space. Remnants of a urinary space (asterisks) are delineated by remnants of the parietal epithelium. In addition to the major proportion of collapsed and/or hyalinized capillaries the sclerotic tuft remnant also contains some patent capillaries (small arrows) suggesting that they were perfused. Filtration or exudation from such capillaries will be directed into the paraglomerular space. At the urinary pole the paraglomerular space extends onto the outer aspect of the tubule (T) by separating the tubular epithelium from its basement membrane, creating a peritubular space (hatched arrows). The disconnected and expanded TBM (long arrows) is clearly visible; it is considered as part of the peritubular space. Both the paraglomerular and the peritubular spaces are separated from the interstitium by a continuous layer of thin sheet-like fibroblast processes (arrowheads). (b) Enlarged view of a urinary pole of another glomerulus with segmental sclerosis (sclerotic tuft portions are not shown; segmental sclerosis was verified in serial sections). At the outside of the parietal epithelium the paraglomerular space extends onto the outer aspect of the proximal tubule (T) by separating the tubular epithelium from its basement membrane (hatched arrows). The paraglomerular and peritubular spaces are separated from the cortical interstitium by a continuous layer of thin sheet-like processes of fibroblasts (arrowheads). The disconnected TBM (long arrows) is considerably thickened and shows a pronounced serrated configuration, suggesting that it first expanded and was subsequently disconnected. FHH rat, 9-month-old. TEMs: (a) × ~525; (b) × ~675.
Fig. 3. Concurrent progression of glomerular and tubular degeneration. Series of 1 μm sections. Sections at the beginning (near the urinary pole) and the end of the series (near the vascular pole, VP) are shown (a and f), respectively. The tuft of this glomerulus is attached to Bowman's capsule by multiple adhesions (asterisks) which begin to merge. The 'sclerotic' adherent tuft portions are enclosed in a paraglomerular space which surrounds the entire glomerulus (beginning in a ending in f). The paraglomerular space is separated from the interstitium by a continuous layer of fibroblasts (arrowheads). Towards Bowman's space the paraglomerular space abuts to the sclerotic tuft portions or is separated by intact portions of the parietal epithelium (small arrows). Several portions of the corresponding proximal tubule (1–6; continuity traced in the complete series) are seen starting at the urinary pole (1, seen in b–d), beginning portion (2, seen in a), and subsequent portions (3–6, seen in b–e). The lumen of segments 1, 2 and 3 is patent and outlined by a flattened proximal epithelium; the lumen of segments 4 and 5 is collapsed showing vacuolar degeneration of the epithelial cells; segment 6 has again an open lumen (seen in d). Along the outside of the urinary orifice (shown by hatched arrows in d) the paraglomerular spaces extend onto the tubule by separation of the TBM from its epithelium (seen in b–d; long arrow in e), creating peritubular spaces. These spaces extend continuously along subsequent tubular segments, later represented by an expanded TBM (b, d and e; long arrows). Transition from the wide tubular spaces created by disconnection of the TBM from its tubular epithelium into those created by the expansion of the TBM is clearly seen in segment 3 (b and c). The expanded TBM surrounding the collapsed segments 4 and 5 is heavily wrinkled. Like the paraglomerular spaces, the peritubular spaces are separated from the cortical interstitium proper by a layer of fibroblast processes. MN rat, 14-month-old. LMs: × ~340.

spreads alongside proximal tubules of nephrons with some maintained glomerular filtration.

This view is supported by the fact that one can find stages of glomerular degeneration which quite obviously represent intermediates between segmental glomerulosclerosis and fully developed glomerular cysts. Figure 7a shows a comparably small tuft adhesion. The degree of damage of this glomerulus has been assessed in a series of sections which showed that the major portion of the tuft is intact and protrudes into
Fig. 4. Stages of tubular degeneration. (a) Fairly normal profile of a proximal tubule. Apart from cell debris in the lumen the only change is the enormously thickened basement membrane surrounded by an almost continuous layer of thin fibroblast processes (arrowheads). Note the serrated configuration of the TBM (arrows). The thickening of the basement membrane is less prominent at a site where a capillary (C) directly abuts the basement membrane. (b) Tubular profile with expanded lumen. The continuous flat epithelium still shows remnants of a brush border pointing to its origin from a proximal tubule. The epithelial tube is enclosed within a thick-walled cylinder of a fairly homogenous extracellular matrix which may be regarded as a dramatically expanded basement membrane. This thick-walled matrix cylinder is surrounded by a complete cover of thin fibroblast processes which, at some sites (arrowheads), are piled up to form several layers. Note the abundant fibrous tissue surrounding the tubular profile. (c) Collapsed tubular profiles surrounded by fibrous tissue. The collapsed epithelial cell cores of the tubules are surrounded by wrinkled cylinders of extracellular matrix covered on their outer aspects by a continuous layer of fibroblast processes (arrowheads). At two sites (arrows) fibroblasts appear to penetrate into the otherwise homogenous matrix. Note the abundance of fibrous tissue surrounding these profiles. (d) Tubular profiles made up of wrinkled matrix cylinders contain some sparse remnants of epithelial cells. In between the wrinkled matrix mantle and the epithelial cell remnants less densely staining spaces are seen which obviously arose from collapse of the tubule with partial disappearance of the epithelium. Again, on their outer aspect these matrix cylinders are covered by layers of thin fibroblast processes (arrowheads). At some sites (arrow) fibroblasts appear to disrupt this layer by penetrating into the core of such profiles. Note many patent capillaries within the surrounding fibrous tissue (asterisks). MN rat, 14-month-old. TEMs: (a) × ~1550; (b) × ~850; (c) × ~1050; (d) × ~600.

an intact Bowman’s space, while the paraglomerular space already extends across the urinary pole onto the proximal tubule. Thereby as usual, the tubular epithelium is separated from its basement membrane; in this nephron the lumen of the tubule is still patent. Figure 7b shows a glomerulus, again with a comparably small tuft adhesion and a perfused intact tuft portion (verified in serial sections), again with extension of the paraglomerular space onto the tubule; in this case, the initial loop of the proximal tubule is entirely surrounded by a common peritubular ‘sack’. Tracing in serial sections revealed that the tubular
Fig. 5. Global glomerulosclerosis associated with the degeneration of the corresponding tubule as seen in a series of 1 μm sections. The tuft is totally sclerotic; it consists of collapsed and/or hyalinized capillaries and mesangial areas; the urinary space has disappeared. Few cells are left inside the former GBM, even fewer outside; these latter cells appear to be remnants of parietal cells (c, d; small arrows). Some narrow capillary lumina contain single erythrocytes or less densely staining material, presumably including plasma proteins (arrowheads). Nevertheless, a continuous vascular route through this collapsed tuft could not be traced in the section series. The efferent arteriole (b, E) appears obstructed. Compared to the glomeruli shown in Figures 2 and 3, an expanded paraglomerular space is missing. The entire profile is tightly surrounded by fibrous tissue. In the vicinity, the corresponding tubuli are seen, including the former transition of the parietal epithelium into the proximal tubule (d; long arrow). All tubular profiles are in an advanced stage of degeneration; most of them consist of solid epithelial cell strands surrounded by thick wrinkled cylinders of extracellular matrix (some of those are marked by stars); others have lost an epithelial core showing up as empty wrinkled mantles of matrix (asterisks). The tuft profile shown (a; peripheral section through this tuft remnant) is marked by a dotted line; in a usual paraffin section such a glomerular remnant would appear as part of interstitial fibrosis. VP, vascular pole; FHH rat, 12-month-old; LM series: ×~375.

When tracing fully developed cysts in serial sections (Figure 8) one regularly encounters a vascular pole with afferent and efferent arterioles which are connected to the tuft remnant. This suggests that the capillaries in this tuft remnant are perfused. In contrast,
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We had the opportunity to study the development of glomerulosclerosis in a kidney which had been surgically removed because of a tumour. In the portion unaffected by the tumour, classic FSGS was found in about one third of the glomeruli. As verified by tracing in serial sections, development of glomerulosclerosis was consistently associated with adhesion of the tuft to Bowman’s capsule. Small tuft adhesions with overt hyalinosis in the adherent tuft portion were seen in the immediate vicinity of fairly intact as well as globally sclerosed glomeruli. Segmental injury of the glomerulus shown in a series of successive profiles in Figure 9 consists of an extensive tuft adhesion to Bowman’s capsule. The ‘sclerotic’ portions of the tuft are massed outside Bowman’s capsule and enclosed within a paraglomerular space. Alongside the urinary pole the ‘sclerotic tissue’ encroaches upon the corresponding tubule which is totally collapsed. Epithelial remnants are surrounded by a thick-walled cylinder of acellular extracellular matrix. An ‘intact’ tuft remnant protrudes into a moderately dilated Bowman’s space.

The glomerular profile shown in Figure 10 is obviously a case of diabetic glomerulopathy as documented by the characteristic matrix nodules. The central part of the tuft appears to be still functional, whereas peripheral parts adhere to the interstitium on a broad front. Outside of Bowman’s capsule the sclerosing process encroaches onto the proximal tubule; similar to what is seen in the experimental models, the sclerotic tissue is apparently separated from the interstitium proper by a layer of fibroblasts. The epithelium of the proximal tubule is thin, no thicker than the parietal epithelium of the remnant of Bowman’s capsule.

Discussion

Focal segmental glomerulosclerosis is consistently associated with tubular degeneration and interstitial fibrosis, altogether, accounting for the progressive decline in renal function. The mechanisms which link glomerular injury to tubulo-interstitial fibrosis are controversial. The present study describes the step-by-step sequence of histopathological events, i.e. the evolution of the injury from the initial lesion in the glomerulus to total nephron destruction. This sequence strongly suggests a novel pathomechanism by which the various stages of injury probably have developed, namely misdirected filtration–exudation from extraterritorial glomerular capillaries toward the interstitium. By ‘extraterritorial’ we refer to their location within a paraglomerular space outside Bowman’s capsule. In previous studies we showed that an initially circumscribed tuft adhesion, progressively enlarges due to ‘misdirected filtration’ towards the interstitium, with the filtrate or exudate spreading between the parietal epithelium and its basement membrane creating a paraglomerular space [8]. Later the parietal basement membrane dissolves; the paraglomerular space...
Fig. 7. Glomerular profiles which can be considered as potential intermediates in the development of glomerular cysts. The accompanying drawings illustrate some details: intact tuft portions are hatched, sclerotic tuft portions are shown in a bird feet pattern, parietal and tubular epithelia are densely stippled, paraglomerular and peritubular spaces are loosely stippled, Bowman’s space, tubular lumina and patent capillary lumina within sclerotic tuft portions are shown in white; the fibroblast border to the interstitium is indicated by a hatched line. (a) Glomerular profile with a comparably small tuft adhesion and a large intact tuft portion. A paraglomerular space broadly associated with the adhesion surrounds the entire profile, extends onto the tubule, and separates the tubular epithelium from its basement membrane (hatched arrow). Compared to many other profiles the encroachment of the degenerative process onto the tubule is more advanced than to the degeneration of the corresponding glomerulus itself. Note the complete separation of the paraglomerular space from the interstitium by a layer of fibroblast processes along the entire circumference of this glomerulus (arrowhead). (b) Glomerular profile with a broad tuft adhesion associated with the prominent paraglomerular space which extends onto the tubule surrounding in total a tubular coil. As verified in subsequent sections, the tubular lumen is obstructed at two sites. A comparably small ‘intact’ tuft portion protrudes into an expanded Bowman’s space. One can assume that the hindered outflow of the filtrate via the obstructed tubule has caused expansion of Bowman’s space. Note, that paraglomerular and peritubular spaces are completely separated from the cortical interstitium by a layer of fibroblast processes (arrowheads). FHH rat, 9-month-old. LMs: × ~400.

becomes separated from the interstitium proper by a layer of sheet-like fibroblast processes. We also showed that the progression from segmental to global sclerosis in a particular glomerulus may either occur by merging of multiple tuft adhesions [9] (see also Figure 3) or by propagating from one lobule to the others via the vascular pole [8]. The latter route is preceded by the extension of paraglomerular spaces onto the glomer-
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Fig. 8. Glomerular cyst, as seen in a series of 1 μm Epon sections. The cyst contains an ‘intact’ tuft remnant with a patent capillary network, supplied by an afferent (c; A) and drained by an efferent (b; E) arteriole. a and d show empty portions of the cyst near the beginning and the end of the section series. The cyst lacks a urinary pole. Obviously, there is no escape route for a filtrate. Apart from the vascular pole region the cyst wall is uniform. It is lined by a very thin but complete layer of parietal epithelial cells followed by a thick homogenous envelope of dense extracellular matrix. Its outer aspect is covered by a layer of fibroblast processes (verified by TEM), and finally surrounded by fibrous tissue. In the vicinity of this cyst the tubular remnants consist of collapsed wrinkled matrix mantles (asterisks). MN rat, 14-month-old. LM series: × ~250.

We conclude that this represents a degenerative pattern of general relevance across species.

In detail, the degeneration of a glomerulus and the corresponding tubule appear to proceed in the following fashion. Initially, the misdirected filtrate is delivered onto the surface of segmentally injured glomeruli into a paraglomerular space. The walling off by a sheet of thin fibroblast prevents the filtrate dissipating into the surrounding interstitium. The filtrate is forced to spread over the outer surface of the glomerulus, eventually comprising the entire glomerulus associated with the progression to glomerulosclerosis. At the urinary pole the cylinder of the TBM provides an outlet. The TBM represents a hydrated, highly water-permeable cylinder; this allows the filtrate to spread within the TBM. In response to the throughput of fluid the TBM swells. Fibroblasts gather around the tubule establishing a tight cover of fibroblast processes on the outer aspect of the TBM. This cover appears to prevent (or at least to decrease) dissipation of fluid from the TBM into the peritubular interstitium. As a consequence, the fluid, which is continuously entering this route at the urinary pole of the glomerulus, will spread within the TBM to evermore distant tubular portions. This process may eventually comprise the entire convo-
Fig. 9. Renal tissue of a patient with focal segmental glomerulosclerosis (series of 3 μm paraffin sections). Accompanying drawings illustrate some details: intact tuft portions are hatched, sclerotic portions together with paraglomerular and peritubular spaces are shown in a bird feet pattern, parietal and tubular epithelia are densely stippled, Bowman’s space is shown in white; the border to the surrounding fibrous tissue is indicated by a hatched line. The glomerulus consists of an ‘intact’ tuft portion protruding into Bowman’s space which is lined by a thin but complete layer of parietal epithelium. The sclerotic tuft portion consists of a broad synechia associated with a large paraglomerular space filled with homogenous, largely acellular, matrix. This space surrounds the entire glomerulus and is separated from the interstitium by a layer of fibroblasts. At the urinary pole the sclerotic tissue and paraglomerular space extend onto the remnants of the proximal tubule. The tubular lumen appears to be totally obstructed. Tubules in the vicinity of this glomerulus either show up as more or less rounded structures entirely filled with a homogenous matrix (stars), or as patent tubular profiles, encircled by rings of extracellular matrix (asterisks). Tumour nephrectomy, unaffected renal parenchyma. Paraffin section, trichrome staining after Goldner, × ~ 240.
Fig. 10. Focal segmental glomerulosclerosis in a patient with diabetic nephropathy. An accompanying drawing illustrates some details: intact tuft portions are hatched, sclerotic portions together with paraglomerular and peritubular spaces are shown in a bird feet pattern, parietal and tubular epithelia are densely stippled, Bowman’s space is shown in white; the border to the surrounding fibrous tissue is indicated by a hatched line. The glomerular tuft contains characteristic Kimmelstiel–Wilson nodules (asterisks). In addition, the tuft forms an extended synechia. The sclerotic tissue fills an enormous paraglomerular space which encroaches upon the tubule. This space is clearly distinguished from the interstitium proper by (i) the much higher content of an acellular, green staining extracellular matrix and (ii), at least at some sites, by fibroblasts lining along the border (arrowheads). The epithelium of the proximal tubule is very thin hardly thicker than the remnants of the parietal epithelium in the glomerulus. Autopsy material, paraffin section, Goldner’s trichrome staining, × ~240.

lution of the proximal tubule. The essential part of our postulate is that after the entire circumference of the TBM has been covered by fibroblast processes, these act as a barrier preventing escape of fluid into the interstitium. Towards the tubule lumen, the epithelium represents a barrier. Thus, the expanded TBM, a cylindrical mantle in shape, has become a water-permeable route which allows the filtrate to spread alongside the proximal tubule.

The local mechanisms accounting for the final collapse and degeneration of the tubule are unknown. This question must be investigated experimentally. A variety of mechanisms may be involved. At the urinary pole (where the quantity of a misdirected filtrate is highest) the expanded basement membrane separates from its epithelium creating the apparently empty, merely fluid-filled spaces directly surrounding the epithelial tube. Thereby, the epithelium is deprived of its basal support. Centripetally directed hydrostatic pressure gradients may lead to compression of the epithelial tube and obstruction. Epithelial cell functions, especially transepithelial transport, may be impaired: (i) due to hypoxia because the distances for O₂/CO₂ diffusion through the expanded TBM and dilated peritubular spaces are increased; (ii) as a result of the interposition of an additional barrier created by the fibroblast sheath between the tubule and the interstitium. Be this as it may, the affected tubules finally collapse and degenerate.

The final degeneration of the glomerulus and the
degeneration of the corresponding tubule may proceed along two different pathways, schematically shown in Figure 11. In most cases (pathway I; Figure 11a–d) progression of segmental glomerular injury to global sclerosis is ahead of tubular degeneration or occurs more or less concurrently with the degeneration of the corresponding tubule. Both glomerulus and tubule finally collapse and the expanded fluid-filled paraglomerular spaces shrink and disappear. This appears to occur at the time glomerular perfusion stops; a vascular route through glomeruli destroyed to such an extent (Figures 5, 6) could no longer be traced. The collapsed glomerular remnants consist of extracellular matrices (predominantly derived from former GBM and mesangial matrix) and a few cells decreasing progressively in number. Finally any remnants are replaced by fibrous tissue. The same sequence is seen in collapsed tubules which are also replaced by fibrous tissue.

A second pathway to nephron degeneration (pathway II; Figure 11e–g) leads to cystic atubular glomeruli. The occurrence of glomerular cysts is well known in literature [33]. They develop predominantly in renal diseases starting with primary tubular damage, e.g. after lithium [34] and cisplatin intoxication [35,36] as well as in renal artery stenosis [37]. In such kidneys atubular glomeruli expand to form glomerular cysts and this is found in up to 50% of nephrons. In kidney diseases with primary glomerular injury, atubular glomeruli do occur as well [38] but in much lower frequency, as documented for diabetic nephropathy [33]. The exact pathogenesis in cases with primary glomerular injury is unknown. It is frequently postulated, however, that interstitial fibrosis secondary to a glomerular lesion may affect neighbouring tubuli (e.g. by local ischemia) and initiate a primary tubular event in the neighbouring nephron.

The sequence of events leading from a primary glomerular injury to atubular glomerular cysts that we propose here, is simple though it would never have been predicted if it were not for morphological studies. We propose the following sequence. The primary injury is segmental glomerulosclerosis associated with misdirected filtration into a paraglomerular space. The spreading of this filtrate along the outer aspect of the tubule causes progressive tubular injury. For reasons as yet unknown, progression of tubular damage becomes the leading process resulting in tubular obstruction of a nephron with some residual glomerular filtration. Since the efflux of this filtrate is prevented by the obstructed tubule, a cystic dilation of the glomerulus ensues. The specific details of this process are so far unknown.

Proposed mechanisms linking progressive glomerular injury to tubulo-interstitial fibrosis include: (i) repeating episodes of glomerular injury leading to self-perpetuating production of growth factors and chemokines by glomerular and interstitial cells [17–22]; (ii) post-glomerular malperfusion resulting in local hypoxia [23–27]; and (iii) excessive leakage and subsequent proximal reabsorption of plasma proteins leading to sustained synthesis and release of inflammatory and vasoactive mediators [28–30]. These mech-

Fig. 11. Schematics to show the progression from a small tuft adhesion (a) to global sclerosis and tubular degeneration: pathway I (b–d) leads to a parallel degeneration of glomerulus and tubulus, pathway II (e–g) to atubular glomerular cysts. Smooth muscle cells, extraglomerular mesangial cells, mesangial cells proper, and endothelial cells are hatched; podocytes are shown in blue, parietal and tubular cells in red, the GBM and the TBM are shown in black, the PBM is shown by thin black lines to indicate its multilayered organization; extraglomerular mesangial cells, mesangial cells proper, and endothelial cells are hatched; podocytes are shown in blue, parietal and tubular cells in red, the GBM and the TBM are shown in black, the PBM is shown by thin black lines to indicate its multilayered organization; collapsing sclerotic tuft portions are shown in black, hyalinized tuft portions are shown in dark green; paraglomerular and peritubular spaces are either shown in light brown (expanded PBM and TBM) or in yellow (spaces resulting from dissolution of PBM or disconnection of TBM); fibroblasts are shown in light green. (a) Small tuft adhesion with a glomerular capillary being attached to Bowman’s capsule. 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 filtration/eduction (arrow) toward the interstitium. The fluid spreads within the parietal basement membrane (PBM) leading first to an expansion of the intrabasement membrane space (shown in light brown) later to the dissolution of the PBM and formation of a paraglomerular space (shown in yellow). (b) Fully developed glomerulosclerosis. Via the vascular pole the adhesion has spread to another lobule. Filtration towards the cortical interstitium has created a tremendous paraglomerular space, which contains the sclerotic tuft remnants, i.e. collapsed or hyalinized GBM-formations. Towards the cortical interstitium the paraglomerular space is separated by a layer of sheet-like fibroblast processes. At the urinary pole the paraglomerular space extends onto the tubule and leads to the formation of peritubular spaces first by expanding of the TBM, later by separation of the expanded TBM from its epithelium. (c) The degenerative process comprises almost the total glomerulus with few capillary loops (one is shown) staying perfused. A remnant of the urinary space is shown containing the cell debris left from podocyte. All sclerotic tuft portions are enclosed in a tremendous paraglomerular space. The peritubular space has extended to subsequent tubular portions by expanding the TBM; the separation of the TBM from its epithelium is restricted to the beginning portions of the proximal tubule. The tubular lumen has become partly obstructed, the tubule as a whole starts to collapse. Paraglomerular and peritubular spaces are separated from the interstitium by a continuous layer of fibroblasts. (d) Globally sclerotic glomerulus with the collapse of the paraglomerular space resulting in the merger of the collapsed tuft remnants and the surrounding fibroblasts. The proximal tubule is in total collapsed, consisting of a thick walled wrinkled cylinder of the expanded former basement membrane surrounded by fibrous tissue; at some sites epithelial remnants are still seen. (e) Beginning of pathway II. Damage progression at the tubule is ahead of that at the glomerulus. The glomerulus contains a comparably small tuft adhesion (segmental sclerosis). However, at the urinary pole the injury has advanced to the formation of an extensive peritubular space by expansion of the PBM and TBM and by separation of both from their respective epithelia. (f) Collapse of the tuube has led to the obstruction of the tubule lumen; consequently, the efflux of the glomerular filtrate from Bowman’s space is prevented. As a consequence Bowman’s space dilates. Moreover, filtration into the paraglomerular space appears to decrease initiating the collapse of the paraglomerular space. (g) Glomerular cyst. The degenerated tubule is disconnected from the glomerulus; a urinary orifice has disappeared. The glomerular cyst consists of a tremendously dilated Bowman’s space outlined by a thin parietal epithelium followed by an expanded solidified paraglomerular space (the former PBM), followed by fibrous tissue. The glomerular tuft is reduced to few loops which are connected to a vascular pole with afferent and efferent arterioles; the capillaries of this ‘mini-tuft’ are perfused. The former area of segmental sclerosis has become part of the cyst wall. The sclerotic formations are contained within the cyst wall; they have decreased in quantity and extension. Tubular profiles in the vicinity show up as wrinkled matrix cylinders of the former basement membrane mostly void of any epithelial remnant.
organisms are not mutually exclusive but may act in an additive manner.

The mechanism proposed here is fundamentally different. The question arises whether this mechanism may interact with the above ones. A major issue concerns the genesis of the peritubular matrix cylinders, i.e. whether they arise from some other mechanism than proposed here, particularly from de novo synthesis of extracellular material by interstitial or epithelial cells. Our immunocytochemical studies do not support synthesis by interstitial fibroblasts. In early stages of tubular injury, when tubules are still patent, the matrix cylinders ensheathing the tubuli do not contain any collagen type I, but contain collagen type IV and laminin, i.e. the ordinary compounds of basement membranes (data not shown). Furthermore, there is no morphological evidence of increased anabolic activity and protein synthesis by tubular epithelial cells; rather, epithelial cells undergo progressive degeneration.

On the other hand, there is undoubtedly marked interstitial cell proliferation around degenerating tubules. The thin cellular sheaths enwrapping the tubules are obviously formed by fibroblasts. Moreover, fibrous tissue accumulates progressively in the surroundings of such tubules and finally replaces them. We think that this most probably occurs secondary to misdirected filtrate spreading and tubular degeneration. Entry of such fluid into the interstitium could trigger synthesis of mediators initiating proliferation. We do not find morphological evidence for increased protein reabsorption in proximal tubules, i.e. prominent endocytotic activity. Moreover, we found no indications of impaired perfusion of these areas. In contrast, the capillary density in intertubular proliferating fibrous tissue is remarkably high, as is well seen in the perfusion-fixed tissue of this study. Taken together, we cannot exclude the possibility that, at least in certain phases of damage progression, the above mentioned hypothetical pathogenetic mechanisms act in concert with the mechanism proposed here. However, in the models investigated in the present study, there were no indications that this actually occurred. Misdirected filtration could be a powerful mechanism to initiate and to maintain progression of interstitial damage and it may well be the dominating process.

There are further arguments to support this notion. In the uninephrectomy-DOCA-SALT model, nephrons degenerate along two quite different pathways [6]. First, FSGS develops and is followed by tubulointerstitial fibrosis as in all the above mentioned models. Second, glomeruli degenerate subsequent to obstruction of preglomerular vessels resulting, early in the disease, in the global collapse of the corresponding glomerular tuft and tubule. Interstitial fibrosis in the vicinity of such nephrons develops without any formation of matrix cylinders around the collapsed tubules (unpublished results). This is in agreement with our view that such cylinders develop from misdirected filtration which is dependent upon segmental merging of the tuft with the interstitium and upon maintenance of the perfusion of the adherent tuft portions. Nephrons with obstruction of pre-glomerular vessel do not develop segmental tuft adhesions and do not preserve perfusion of the degenerating glomerular tuft. Furthermore, experimental data (to be published elsewhere) document the spreading of tracers from injured glomeruli into paraglomerular spaces and along corresponding tubuli. These observations strongly support the concept presented here.

The histopathological findings in patients with ‘classic’ FSGS resemble those in experimental models of FSGS [39,40]. Apparent differences when comparing the material of the present study (compare Figures 3 and 9) appear to be explained mainly by difference in the histological techniques: Figure 3 originates from a 1 μm section of perfusion fixed, plastic embedded tissue, whereas Figure 9 originates from an immersion fixed, routinely prepared paraffin section. In the human material the ‘sclerotic tissue’, which fills the paraglomerular spaces and extends into the peritubular spaces appears to be more ‘solid’ than in the rat. In humans, development and progression of sclerosis are apparently slower than in the rat and this is in accordance with the different life spans of rats and man [41]. The layered appearance of the sclerotic tissue around degenerating glomeruli remnants is possibly due to discontinuous damage progression. If this interpretation is correct, the lesions would reflect repeated episodes of injury and repair. This may also explain why in humans the process progresses slower and why histopathological examination frequently suggests consolidation rather than progression of glomerulosclerosis.

In this context a further point should be discussed. Several studies analysing the histopathological changes in chronic renal disease have shown that the severity of tubular interstitial fibrosis correlates more closely with the loss of glomerular filtration rate than does the severity of glomerulosclerosis [42–46]. These results have been interpreted to indicate that, at least in some forms of chronic renal disease, interstitial proliferation and matrix deposition are the leading processes linking glomerulosclerosis as a secondary event to interstitial fibrosis as the primary process. This conclusion is not in agreement with the mechanism proposed here. The morphometric studies which suggested that the severity of tubular interstitial fibrosis rather than that of glomerulosclerosis correlates with the loss of GFR fail to provide one crucial piece of evidence. To show unambiguously that interstitial fibrosis is the primary mechanism, it would be necessary to know the absolute number of remaining functional glomeruli. Studies in the ageing kidney [47–50] suggest that glomeruli may completely vanish. The sites of former glomeruli show then up as sites of interstitial fibrosis. In the uninephrectomy-DOCA-SALT model, nephrons degenerate rather than that of glomerulosclerosis correlates with the loss of GFR fail to provide one crucial piece of evidence. To show unambiguously that interstitial fibrosis is the primary mechanism, it would be necessary to know the absolute number of remaining functional glomeruli. Studies in the ageing kidney [47–50] suggest that glomeruli may completely vanish. The sites of former glomeruli show then up as sites of interstitial fibrosis (Figure 5). Such fibrous replacement of scarred glomeruli increases the interstitial damage score and decreases the glomerular damage score (or will leave it constant, at best). Without quantitation of the number of remaining glomeruli, calculation of this correlation makes little sense.

Taken together, the present study suggests that
severely injured glomeruli may continue to filter with the filtrate spreading along interstitial routes. Even if nothing is known about the haemodynamics of glomerular capillaries perfusing a synechium, it may readily be suggested that the high hydrostatic pressure and the specific permeability characteristics of glomerular capillaries are maintained in these capillaries as well. Thus, the fluid added locally to the interstitium from such 'extraterritorial' glomerular capillaries probably is quite different in quantity and composition compared to that from interstitial capillaries. We propose that this kind of abnormal addition of fluid is the essential mechanism accounting for interstitial progression of the disease. Experimental work is necessary to elucidate the particular effects of such misdirected filtration into the interstitium.

Acknowledgements. We thank Ingrid Hartmann for technical assistance, Ingrid Ertel for the photographic work, Marlis Schuchardt for secretarial assistance; Rolf Nonnenmacher performed the excellent art work. Prof. Dr H. E. Otto (Department of Pathology, University of Heidelberg), Prof. Dr M. J. Mieths (Department of Pathology, University of Basel) and Prof. Dr S. Peter (Department of Urology, Städtisches Krankenhaus Darmstadt) provided tissues from human cases of chronic renal disease; we greatly appreciate their help. The Milan normotensive rats have kindly been given by Prof. Dr G. Bianchi (Ospedale San Raffaele, Milano). This study was supported by Deutsche Forschungsgemeinschaft, project Kn 546/9–2.

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Received for publication: 29.6.98
Accepted in revised form: 15.7.98