

The renal cortical interstitium: morphological and functional aspects

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Abstract The renal interstitial compartment, situated between basement membranes of epithelia and vessels, contains two contiguous cellular networks. One network is formed by interstitial fibroblasts, the second one by dendritic cells. Both are in intimate contact with each other. Fibroblasts are interconnected by junctions and connected to basement membranes of vessels and tubules by focal adhesions. Fibroblasts constitute the “skeleton” of the kidney. In the renal cortex, fibroblasts produce erythropoietin and are distinguished from other interstitial cells by their prominent F-actin cytoskeleton, abundance of rough endoplasmic reticulum, and by ecto-5'-nucleotidase expression in their plasma membrane. The resident dendritic cells belong to the mononuclear phagocyte system and fulfil a sentinel function. They are characterized by their expression of MHC class II and CD11c. The central situation of fibroblasts suggests that signals from tubules, vessels, and inflammatory cells converge in fibroblasts and elicit an integrated response. Following tubular damage and inflammatory signals fibroblasts proliferate, change to the myofibroblast phenotype and increase their collagen production, potentially resulting in renal fibrosis. The acquisition of a profibrotic phenotype by fibroblasts in renal diseases is generally considered a main causal event in the progression of chronic renal failure. However, it might also be seen as a repair process.

Keywords Myofibroblasts · Dendritic cells · Unilateral ureter obstruction · Thiazide diuretics · Morphology

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Introduction

In all parenchymal organs, including the kidney, the interstitium is situated in the space between the basement membranes of the epithelial cells and of the nutritive capillaries (Fig. 1). This compartment is of eminent functional relevance in the healthy and diseased kidney.

The fibroblasts in the interstitium provide the “skeleton” of the tissue and maintain the three-dimensional architecture of the tissue. The interstitium is necessarily involved in all intrarenal exchange processes since the reabsorption and secretion of fluid and solutes implicates a transit across the interstitial compartment. Cortical fibroblasts play a role in the production of regulatory substances, such as extracellular adenosine (Le Hir and Kaissling 1993) and erythropoietin (Bachmann et al. 1993; Maxwell et al. 1993). In the healthy organ dendritic cells, belonging to the mononuclear phagocyte system, reside in the interstitium in line with their sentinel function. In disease, various inflammatory cells infiltrate the interstitium. In the diseased kidney, the cellular content as well as the content of fibers and matrix in the interstitium increases, eventually yielding renal fibrosis.

The cortical interstitium, and in particular the fibroblasts, are often overlooked in conventional tissue preparations of healthy kidneys. However, under inflammatory conditions the interstitial space becomes massively expanded and fibroblasts acquire the myofibroblast phenotype: they express alpha smooth muscle actin (α SMA) and have an increased capacity for production of collagen fibrils and matrix.

In this review based on studies in rats and mice we describe the morphology of interstitial cells of the healthy renal cortex and in various models of diseases. The observations suggest that fibroblasts are the pivotal cells in the renal interstitium.

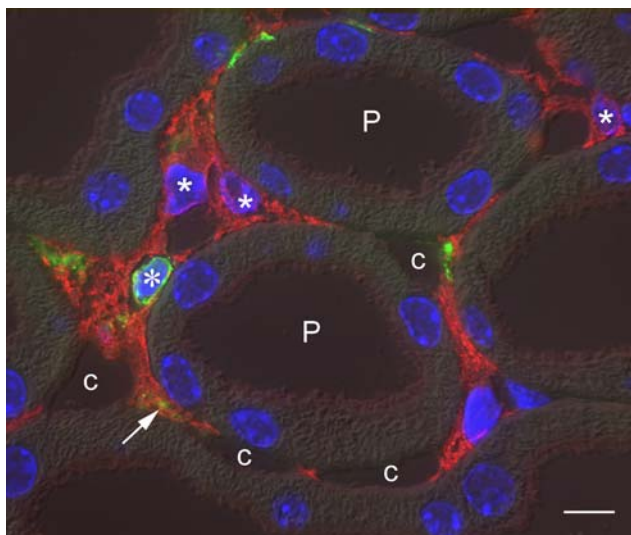


Fig. 1 Cortical peritubular interstitium in a mouse kidney (3 μ m cryostat section). The interstitial space extends between tubules and capillaries (*c*). It is occupied by fibroblasts (*stars*) and their processes, expressing 5'NT (*red*) and dendritic cells (*asterisks*), expressing MHC class II (*green*). Cellular nuclei are labeled in *blue*; the tissue structure is shown by differential interference contrast (DIC). The brush border of proximal tubules (*P*) is weakly labeled by 5'NT. Bar 10 μ m

Problems in studying interstitial cells in the kidney

Following older electron microscopic investigations (Bulger and Nagle 1973; Lemley and Kriz 1991; Takahashi-Iwanaga 1991) only a few reports dealt with the structure of interstitial cells in healthy kidney. The reasons are mostly technical. The study of the cells in the cortical interstitium is difficult since after immersion fixation the interstitial space and the capillaries are usually collapsed whereas tubules are swollen. The light microscopic distinction between fibroblasts, endothelial cells, and dendritic cells is awkward in such tissue preparations. Only perfusion fixation and the use of thin light microscopic (<3 μ m) sections and of electron microscopy provide a reasonably safe distinction of fibroblasts and other interstitial cells.

Identification of cortical fibroblasts by immunostainings

The study of renal fibroblasts is further hampered by the lack of marker proteins expressed in all fibroblasts in all functional stages and exclusively in fibroblasts.

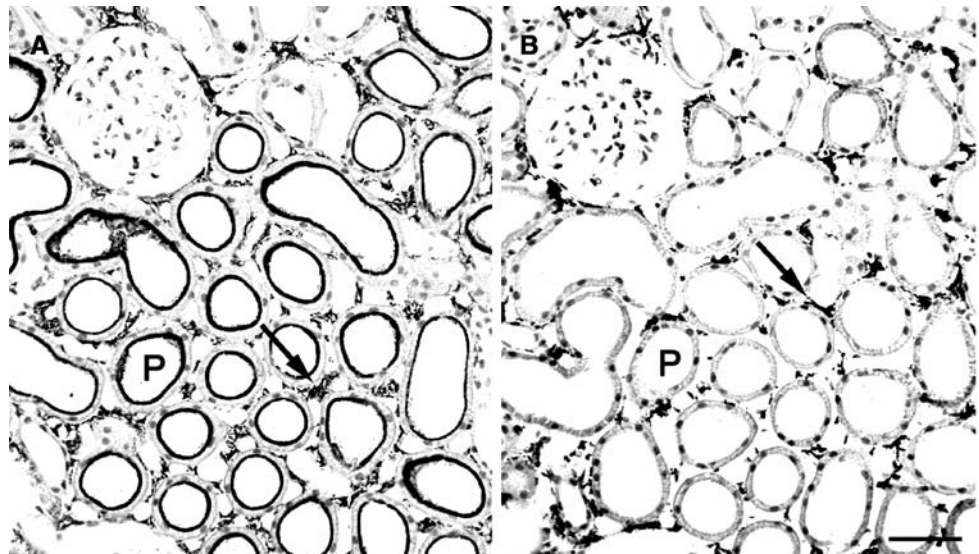
Nevertheless, a couple of proteins have been proposed to identify fibroblasts in healthy and diseased kidneys, the most propagated one being the “fibroblast specific protein 1 (FSP1) (Strutz et al. 1995) which corresponds to the small cytoplasmic calcium-binding protein S100A4 and plays a

role in cell motility (Garrett et al. 2006, 2008). In the healthy kidney only very few cells in the interstitial space are recognized by antibodies against S100A4/FSP1. This number is by far too small to comprehend the entire population of fibroblasts in the healthy kidney. In the diseased kidney, the incidence of S100A4/FSP1-positive cells increases in parallel with the amount of infiltrating leukocytes (Inoue et al. 2005). In fact, in the healthy as well as in the diseased kidney S100A4/FSP1 co-localizes with leukocyte markers (Inoue et al. 2005; Le Hir et al. 2005; Rossini et al. 2005). S100A4/FSP1 also labels smooth muscle cells of arterioles (Le Hir et al. 2005) and metastasing cells (Davies et al. 1993; Gibbs et al. 1994; Mazzucchelli 2002; Garrett et al. 2006). Recently the stromal cadherin 9 (Thedieck et al. 2007) has been proposed as surface marker for fibroblasts in the human kidney. Cadherin 9 is expressed in a very small population of interstitial cells in healthy kidneys. In the diseased kidney the incidence of labeled cells is higher. Cadherin 9 partially co-localizes with cells labeled by FSP1/S100A4 and with mononuclear cells (Thedieck et al. 2007).

Alpha SMA is expressed in fibroblasts of the adult kidney only under pathological conditions. In other words α SMA allows the identification myofibroblasts but not of the fibroblasts of the healthy kidney.

Other proteins that label interstitial fibroblasts are the soluble guanylyl cyclase (sGC) (Theilig et al. 2001) and the membrane-bound ecto-5'-nucleotidase (5'NT) (Dawson et al. 1989; Le Hir et al. 1989; Gandhi et al. 1990). Both proteins recognize interstitial cells (Ito cells) in the liver as well (Schmid et al. 1994; Theilig et al. 2001) and are functionally related with regulation of hemodynamic parameters (Le Hir and Kaissling 1993). In the renal cortex, in addition to peritubular fibroblasts, mesangial cells, and perivascular cells express sGC. The enzyme 5'NT is identical with CD73 (Thompson et al. 1989). In the interstitial space it is strongly expressed in the plasma membrane of cortical peritubular fibroblasts, and weakly in a small subgroup of T-lymphocytes. Furthermore, it is expressed in the brush border membrane of proximal tubular cells and in the apical or basolateral plasma membrane of intercalated cells. In mice 5'NT also labels mesangial cells (Le Hir and Kaissling 1993). The epithelial and mesangial locations of 5'NT do not impair its usefulness as marker for cortical fibroblasts within the interstitium. The occasionally CD73-positive lymphocytes can hardly be mistaken as fibroblasts due to their small size and their rounded shapes. Thus, 5'NT is a reliable marker for identification of renal cortical fibroblasts (Figs. 1, 2). The identification of fibroblasts as source of renal erythropoietin was possible due to the distinct outlining of interstitial fibroblasts by 5'NT (Bachmann et al. 1993; Maxwell et al. 1993, 1997). In a 5'NT-expressing cell line derived from adult renal fibroblasts hypoxic or

Fig. 2 Interstitial cells, highlighted by immunogold staining for ecto-5'-nucleotidase (**a**) and MHC class II (**b**) on consecutive cryostat sections. **a** 5'NT, detected by immunogold, depicts the abundance of interstitial fibroblasts (*arrow*); the brush border of proximal tubules (*P*) is stained too. **b** Immunogold labeling for MHC class II reveals the distribution of dendritic cells (*arrow*) throughout the interstitium. Bar 50 μm



anoxic incubation conditions provoked erythropoietin expression and increased vascular endothelial growth factor expression (Plotkin and Goligorsky 2006).

In contrast with cortical interstitial fibroblasts, fibroblasts of the renal capsule, of the periarterial connective tissue sheath, and of the medulla do not or only weakly express 5'NT.

Developmental aspects of cortical peritubular fibroblasts

The sharing by renal fibroblasts of many proteins ascribed typically to either mesenchymal cells or to epithelial cells might be rooted in the developmental origin of renal fibroblasts. Unlike in other epithelial organs, where epithelial and stromal cells arise from different blastema, the metanephric mesenchyme gives rise to both, epithelia and stroma. Whereas the signaling cascades promoting the transformation of metanephric mesenchymal cells to epithelial cells are extensively studied, little is known about the differentiation of the mesenchymal cells to interstitial fibroblasts (Eklblom and Weller 1991; Alcorn et al. 1999). During embryogenesis part of the metanephric mesenchyme is induced to develop into nephron epithelia. These prospective epithelial cells are surrounded by a population of supporting mesenchymal cells, which express the winged helix transcription factor BF-2. BF-2 seems to determine the fate of this population of mesenchymal cells to maintain the mesenchymal phenotype and to differentiate later to cortical fibroblasts (Hatini et al. 1996). The expression of α SMA apparently depends on signaling by Wnt-4, a member of the Wnt family of secreted signaling molecules. Wnt-4 probably performs this function in the developing kidney by activating the Bmp-4 gene encoding

a known differentiation factor for α SMA (Itaranta et al. 2006).

In newborn rats the stromal cells in the kidney display α SMA, vimentin (Marxer-Meier et al. 1998), and nestin, which is associated with vimentin (Sakairi et al. 2007). Yet, they are all negative for 5'NT, although at that time point the enzyme is well detectable in its epithelial locations. In the latter the “mesenchymal” cytoskeletal proteins (α SMA and vimentin) are downregulated. The cortical peritubular fibroblasts (but not the medullary ones) lose α SMA- and vimentin as well as nestin expression gradually during the first two postnatal weeks, simultaneously with the gradual gain of 5'NT. During a short period within the second postnatal week α SMA, vimentin, and 5'NT are co-expressed by the same cells. Three weeks after birth the peritubular cortical fibroblasts in healthy rat kidneys display the adult fibroblast phenotype, i.e. 5'NT in their plasma membrane, but neither vimentin, nor α SMA in the cytoplasm (Marxer-Meier et al. 1998). The fibroblasts in the juxtamedullary layer of the cortex, containing the most mature, oldest generation of nephrons, are the first to gain the “mature” stage. Interestingly, the fibroblasts in the periarterial interstitial tissue maintain during adult life a moderate α SMA- and vimentin expression associated with an extremely weak or undetectable 5'NT expression (Kaissling et al. 1996; Marxer-Meier et al. 1998). These observations strengthen the notion of the dependence of the fibroblast phenotype on the microenvironment.

Cellular constituents of the healthy cortical interstitium

Renal fibroblasts and dendritic cells (DC) (Figs. 1, 2) form closely enlaced networks in the interstitium. In healthy interstitium other cell types are found only occasionally.

Morphology of fibroblasts

The fibroblasts in the peritubular interstitium (Figs. 2, 3) bridge the spaces between the capillaries and the epithelia (Kaissling and Le Hir 1994) and form a continuous network throughout the kidney. Renal fibroblasts display similar shapes and ultrastructure as fibroblasts in the interstitium of other organs (Sappino et al. 1990; Hashizume et al. 1992; Maxwell et al. 1997).

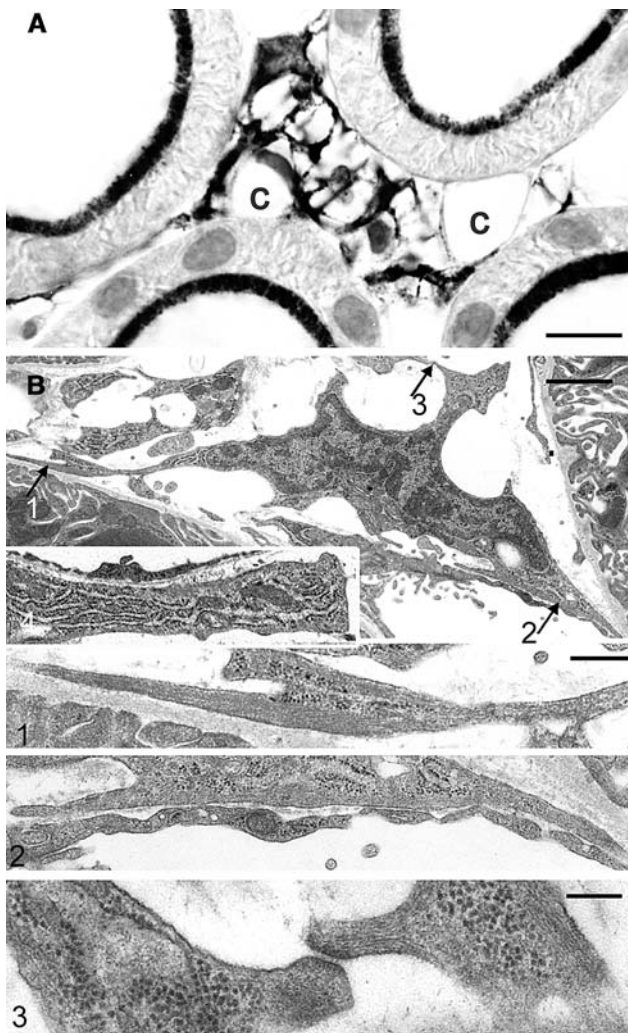


Fig. 3 Fibroblasts in the cortical peritubular interstitium. **a** Cryostat section. Fibroblasts, shown by immunogold labeling for 5'NT, bridge the interstitial space between the tubules and capillaries (c); **b** transmission electron microscopy of healthy rat kidney. Stellate pericaryon of a fibroblast, connected to the basement membrane of a proximal tubule (1, magnified in *insert 1*), the basement membrane of a capillary (2, magnified in *insert 2*) and to another fibroblast process (3, magnified in *insert 3*). Most cell organelles are located in cellular processes but lack in the pericaryon. Note the bundles of F-actin filaments at the connection sites. *Insert 4* rough endoplasmic reticulum in a fibroblast process. Bars **a** 10 μm , **b** 1 μm , inserts 0.25 μm

Skeleton function

In healthy kidneys the pericaryon of the fibroblasts reveals a stellate profile with clear outlines (Figs. 3, 4 and 5). Characteristic for cortical fibroblasts in healthy kidneys is the thin organelle-free cytoplasmic rim around the nucleus and the thin layer of actin filaments immediately under the plasma membrane, well apparent in transmission electron microscopy (Figs. 3, 5) and by staining for F-actin. The cellular processes are connected to the basement membranes by pedicle-like (rats) (Fig. 3) or spine-like (mouse) (Fig. 5) extensions. In these attachments the abundant F-actin filaments are suggestive of stress fibers. In addition to the contacts to basement membranes of capillaries and epithelia the fibroblast processes are also interconnected with each other by intermediate junctions (Fig. 3) (Lemley and Kriz 1991; Kaissling et al. 1996). By bracing the space between vessels, renal corpuscles, and loops of convoluted tubules the fibroblasts form a continuous network through the entire cortex thereby maintaining the tissue architecture.

The complex shape of fibroblasts in the kidney cortex is difficult to comprehend from two-dimensional images of tissue sections. Scanning electron microscopic images (Fig. 4) revealed that the larger fibroblast processes correspond to flat pierced leaves apposed to tubules and partly enveloping other structures, such as dendritic cells (Takahashi-Iwanaga 1991; Ina et al. 2002), whereas filiform processes span longer distances. The adhesions to the basement membranes of tubules and capillaries as well as to other fibroblasts suggest that cross-talk between epithelial cells and fibroblasts might be mediated not only by

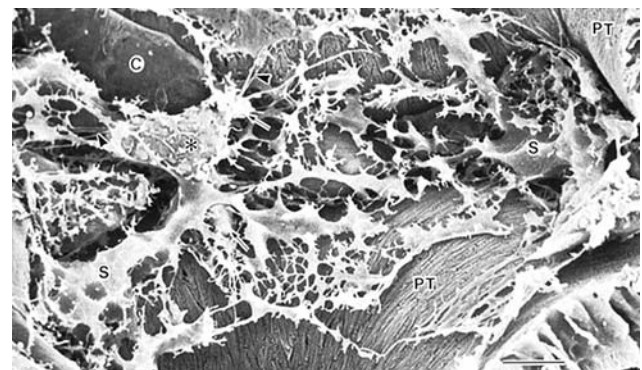


Fig. 4 Three-dimensional view of a fibroblast in the cortical interstitium of a rat. Scanning electron microscopy, after digestion of the tubular and vascular basement membranes. The pericaryon (S) shows characteristic sharp angles; the processes adjacent to the proximal tubule (PT) are extremely attenuated and perforated; the asterisk indicates a dendritic cell enclosed by fibroblast processes, arrowheads indicate processes of the dendritic cell; C capillary. Bar 10 μm . (Image from Takahashi-Iwanaga (1991) Cell Tissue Res 264:269–281)

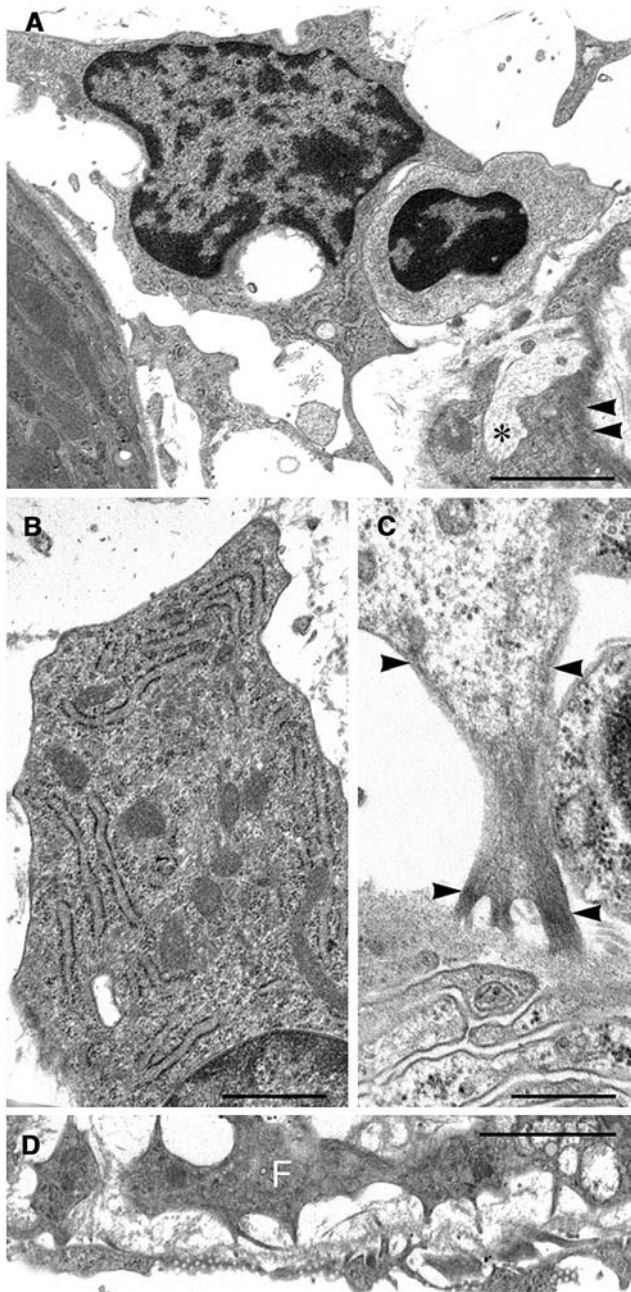


Fig. 5 Fibroblast in the cortical peritubular interstitium of a healthy mouse (transmission electron microscopy). **a** Stellate pericyon with a thin cytoplasmic rim and large processes, and close juxtaposition to a lymphocyte; matrix, released by the fibroblast (*asterisk*); F-actin filaments beneath the plasma membrane (*arrow head*). **b** The abundant rough endoplasmic reticulum (rER), mitochondria and Golgi apparatus are located in the large cell processes; the rER cisterns are densely studded with ribosomes and free ribosomes are frequent. **c** attachment to the basement membranes of tubules and capillaries frequently occurs by spine-like processes, densely filled with F-actin stress fibers (*arrow heads*). **d** Tangential sections of an endothelial cell and part of a fibroblast (*F*), showing the anchoring of the fibroblast to the basement membrane by spine-like processes. *Bars* **a** 4 μm , **b** 1 μm , **c** 0.5 μm , **d** 2 μm

chemokines (van Kooten and Daha 2001) but as well by mechanical forces (Fujigaki et al. 2005).

Protein synthesis apparatus

The synthesis of collagen fibers (types I, III and VI), of unbanded microfilaments and of matrix is part of the scaffolding function by fibroblasts (Lemley and Kriz 1991). This function is mirrored by the prominent apparatus for protein synthesis. Indeed, the extensive rough endoplasmic reticulum (rER) distinguishes fibroblasts from all other interstitial cells (Figs. 3, 5). The abundant meandering and anastomosing cisterns of the rough ER are particularly densely studded with ribosomes and show large profiles, which are filled by a flocculent, rather electron dense material. Free ribosomes are abundant. The rER cisterns are almost exclusively located in the large cytoplasmic processes of fibroblasts that might be quite distant from the pericyon. The Golgi fields are usually located together with the rER in the periphery of the cells, where in electron microscopy the formation of collagen fibrils is often well detectable in the direct vicinity of the plasma membrane. This clearly designates the cell as fibroblast (Fig. 5). In “quiescent” fibroblasts the nucleus shows often angular profiles and reveals large amounts of heterochromatin condensations (Fig. 5), whereas in “activated” stages with increased matrix production the nucleus becomes enlarged, rounded and displays much more euchromatin (see below).

Other cell organelles are situated among the rER cisterns. Mitochondria are numerous. Fibroblasts can accumulate lipid droplets. These are not common in cortical fibroblasts of healthy kidneys; yet, they constitute a constant and characteristic feature of fibroblasts in the medulla, in particular in the inner medulla. They appear also in cortical fibroblasts of anemic kidneys (Kaissling et al. 1993). Lysosomal bodies, which point to a phagocytotic capacity (Lullmann-Rauch 1987; Sundelin and Bohman 1990) are rarely observed under control conditions.

Morphology of dendritic cells

Dendritic cells (DC) belong to the mononuclear phagocyte system, acting at the cross road of innate and adaptive immunity, self-tolerance, and tissue homeostasis (Soos et al. 2006; John and Nelson 2007). Interstitial DCs continually probe the surrounding environment through dendrite extension and readily respond to insults to the parenchyma they survey (Dong et al. 2005; Soos et al. 2006; John and Nelson 2007). DCs have been recognized by their expression of MHC class II (Fig. 6) and have been isolated on account of their membrane expression of CD11c (Kruger

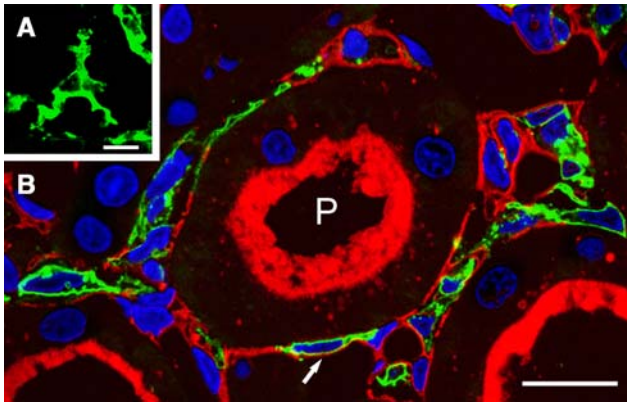


Fig. 6 Dendritic cells in the cortical peritubular interstitium. **a** Here comes the sentinel! dendritic cell (green) in the interstitial space. **b** 1 µm cryostat section; dendritic cells express MHC class II (green), fibroblasts express 5'NT (red), nuclei are stained in blue. Note the juxtaposition of fibroblast processes (arrow) and dendritic cells. *P* proximal tubule with brush border (red). Bar **a** 1 µm, **b** 10 µm

et al. 2004). In the healthy kidney DCs are present in their immature phenotype with comparably low levels of MHC class II and of co-stimulatory proteins (Kruger et al. 2004), but with a high capacity for uptake of antigens (Dong et al. 2005). They lack typical phagocyte markers. Practically, the immuno-labelling for MHC class II or CD11c and in mice also for F4/80 allows easy detection of dendritic cells in the healthy and diseased kidney.

In the kidney, DCs form an organ-spanning network (Soos et al. 2006), which is narrowly intertwined with the fibroblast network (Fig. 2). Thus, dendritic cells and fibroblasts are usually found in strikingly close contact with each other (Figs. 6, 7) (Kaissling and Le Hir 1994; Kaissling et al. 1996). Because DCs constantly form and retract dendritic extensions the shape revealed in a tissue sections represents a “snap-shot”. Usually dendritic cells appear stellate in tissue sections (Fig. 7). Unlike fibroblasts the pericaryon of dendritic cells is large and rounded, and confines a rounded nucleus and most cell organelles. Dendritic cells have, in comparison to infiltrating cells (macrophages, lymphocytes), more mitochondria, more rER, and a large Golgi apparatus (Fig. 7). Lysosomes are less apparent than in macrophages. The so-called Birbeck granules, which are characteristic for dendritic cells, are a special formation of the endocytotic compartment, serving as a loading compartment and/or reservoir of antigens before DC maturation (Fig. 7) (Mc Dermott et al. 2002). The rER of dendritic cells differs from that of fibroblasts. The cisterns are narrow, little ramified, and regionally lacks the dense studding of ribosomes and the lumen of the rER cisterns is electrolucent (Fig. 7). The intermediate filament protein vimentin is regularly present in the pericaryon of DCs whereas it is absent in fibroblasts in the healthy renal cortex.

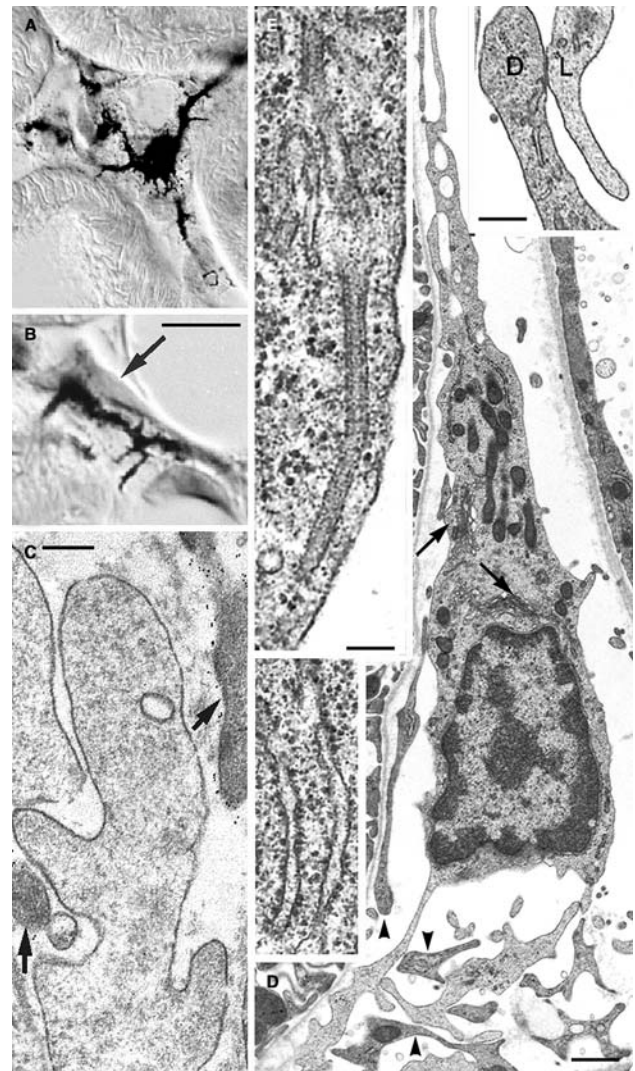


Fig. 7 Dendritic cells in the cortical peritubular interstitium. **a, b** Cryostat sections, immunogold staining for MHC class II, showing dendritic cells, and differential interference contrast (DIC). The processes of the dendritic cells have extensive contact with fibroblasts (arrow). **c** the peripheral processes of dendritic cells are virtually devoid of large cell organelles and appear lighter than the processes of the F-actin filament-displaying fibroblast processes (arrows), here labeled with immunogold for ecto-5'-nucleotidase. **d** The pericaryon displays a rounded nucleus, numerous mitochondria, Golgi apparatus (arrows) and rough endoplasmic reticulum (see insert 1); the cell processes are branched and appear much lighter than those of fibroblasts (arrowheads). Insert 2 adhesion between processes of a dendritic cell and of a lymphocyte. **e** Birbeck granule, in a dendritic cell. Bars **a, b** 10 µm; **c** 0.1 µm; **d** 1 µm; insert 0.5 µm; **e** 0.01 µm

The broad ramified and flat “veil-like” processes, that often appear perforated like laces are largely devoid of cell organelles and they lack the prominent stuffing with F-actin filaments, characterizing fibroblasts. In addition they have long filiform processes, of approximately the same diameter as the filiform fibroblast processes. In transmission electron microscopy a distinction of fibroblast and DC

processes is, however, possible without any marker. Due to the lack of F-actin the processes of dendritic cells appear always much less electron dense than the fibroblast processes (Fig. 7). The lack of the prominent F-actin skeleton is in line with the lack of junctional connections between DCs and tubules or vessels, in contrast to fibroblasts. However, frequently the plasma membranes of dendritic cells and of fibroblasts, or dendritic cells and lymphocytes form spots of membrane adhesions, so-called “kisses” (Fig. 7). Double immuno-staining using 5'NT for fibroblasts and MHC class II for dendritic cells allows an appreciation of the narrow intermingling of both cell types (Fig. 6), suggesting the possibility of extensive cross-talk between them.

Macrophages, lymphocytes and granulocytes

Macrophages and lymphocytes are rarely found in the healthy renal interstitium but they invade the interstitial spaces under inflammatory conditions (Eddy 2005). A large proportion of the invading mononuclear cells display, besides the established marker proteins (e.g. CD 45, CD3, CD4, CD 8; ED1, ED2, CD44), the cytosolic protein S100A4/FSP1 (Le Hir et al. 2005). Neutrophil granulocytes

are found occasionally, basophil and eosinophil granulocytes and plasma cells are rare in the healthy cortical renal interstitium.

Morphology of myofibroblasts

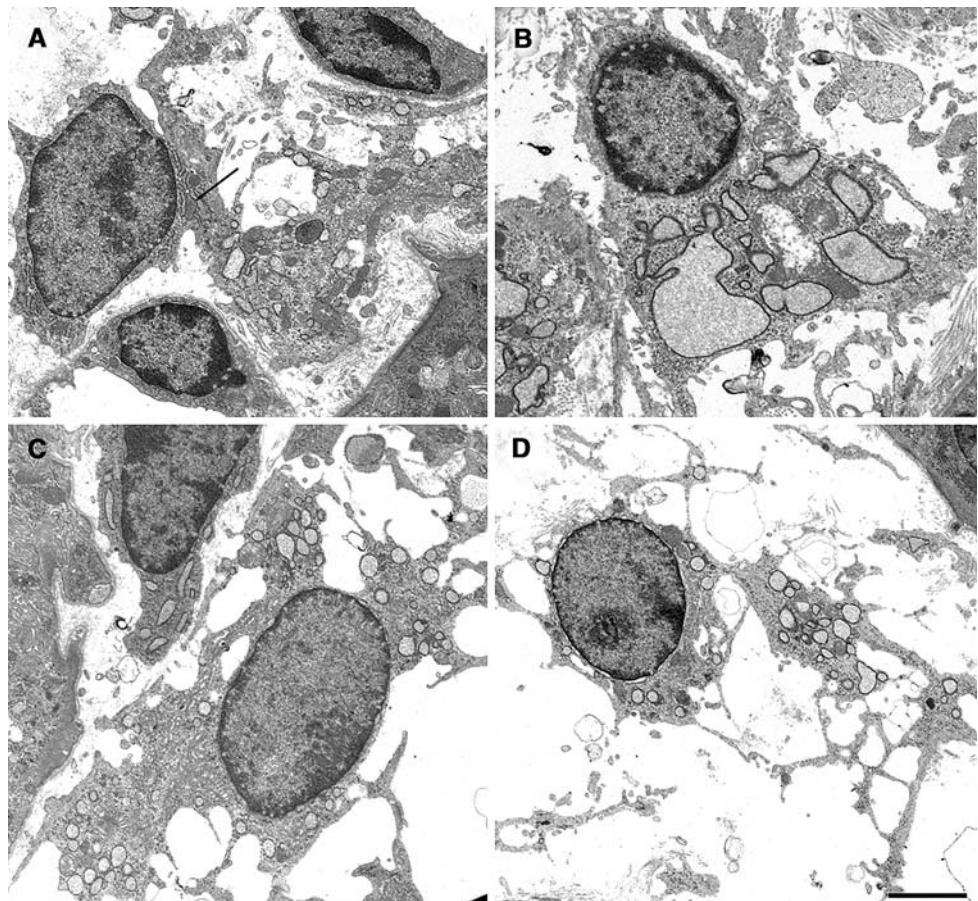
Any chronic inflammation of the interstitium goes along with the appearance of interstitial cells with a specific phenotype, the myofibroblasts. The myofibroblasts are a landmark of renal fibrosis which is the final common pathway for all chronic kidney diseases and is eventually associated with the loss of kidney function.

Functionally myofibroblasts resemble, on one hand, fibroblasts because of their matrix production and, on other hand, smooth muscle cells because of their contractility due to the presence of α SMA.

On account of structural criteria there exists a continuum from “quiescent” fibroblasts to “activated” myofibroblasts and intermediate forms between typical interstitial fibroblasts and myofibroblasts can be found (Picard et al. 2008).

By definition myofibroblasts are large stellate cells, but their appearances are extremely variable (Fig. 8). In contrast to normal fibroblasts their nucleus is generally rounded (Fig. 8) sometimes with indentations, and appears

Fig. 8 Gallery of myofibroblasts (transmission electron microscopy). Myofibroblasts show a heterogeneous morphology. **a** myofibroblasts after 24 h of ureter ligation with transitory features between a “quiescent” fibroblast with narrow rER (around the nucleus) and a typical myofibroblasts with inflated cisterns of rER (in the cellular process); **b** 2 days after ureter ligation with largely inflated cisterns; **c** after 3 days showing a stellate shape; **d** after 3 days of ureter ligation. Bar 2 μ m



lighter than nuclei of “quiescent” fibroblasts, due to less heterochromatin condensations. It is surrounded by a broad cytoplasmic rim. Morphological evidence for increased production of matrix is the abundant rER, most often with inflated cisterns (Figs. 8, 9). The latter are found in the large pericaryon and in the broad, extensively branched cytoplasmic processes. The accumulations of collagen fibrils in cellular indentations, and the occasional presence of an incomplete layer of basement membrane-like matrix on the cell surface manifest the enhanced matrix production by the cells (Fig. 9).

The hallmark of myofibroblasts is the presence of the α SMA (Desmouliere et al. 2005; Hinz 2007; Hinz et al. 2007) which builds bundles of microfilaments with dense bodies (Fig. 9). These bundles may run across the cytoplasm and are not necessarily associated with the plasma membrane, as the F-actin filaments in quiescent fibroblasts, and they are concentrated in cellular processes. The frequency of intercellular attachments by intermediate junctions and of hemidesmosome-like attachments to basement

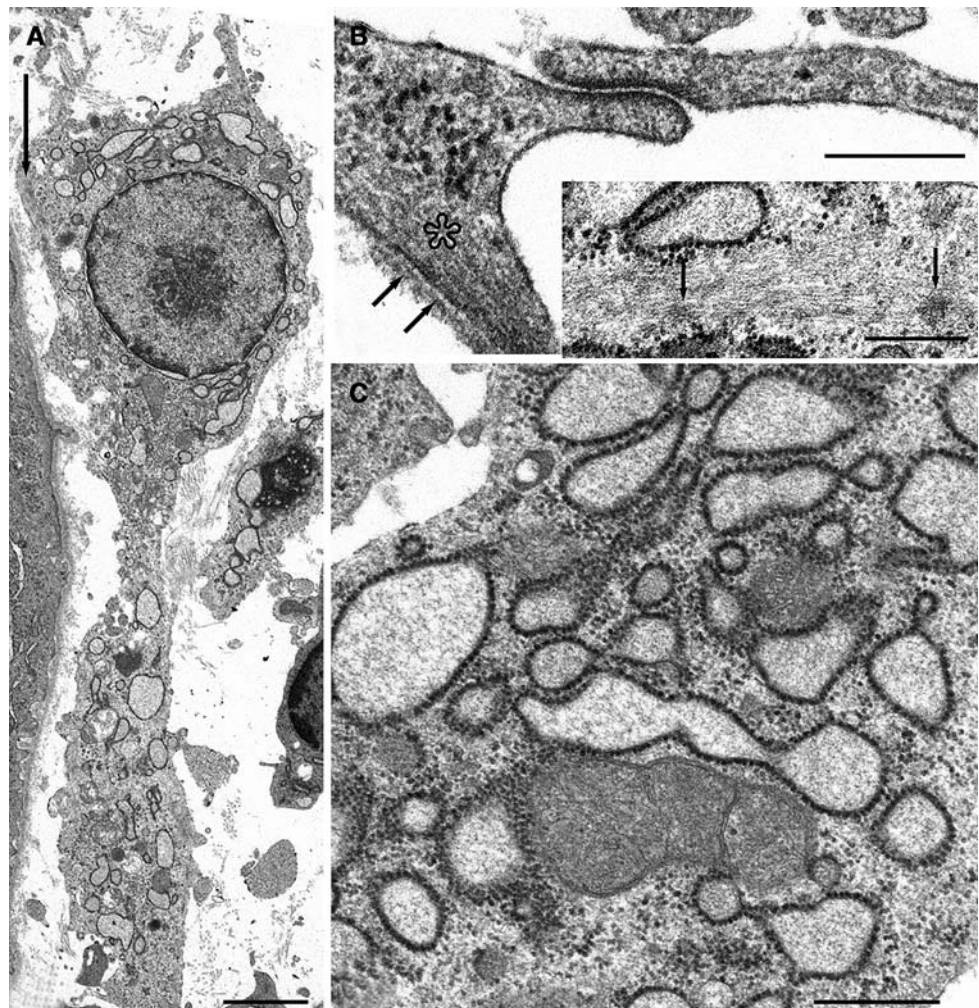
membranes of tubules and vessels is much higher than in quiescent fibroblasts.

Furthermore, the intermediate filament protein vimentin is expressed in myofibroblasts, but not in quiescent fibroblasts of adults. Desmin, another intermediate filament protein, has been described to be present in myofibroblasts (Maxwell et al. 1997); however, we and others (Essawy et al. 1997) never detected desmin in renal myofibroblasts.

Role of fibroblasts in regulation of regional renal hemodynamics and in renal erythropoietin production

The presence of 5'NT in cortical fibroblasts indicates that adenosine can be produced in the cortical interstitium. 5'NT is responsible for conversion of extracellular 5'-AMP to adenosine (Eltzschig et al. 2003). The bulk of extracellular 5'-AMP in kidney most probably originates from tubular cells and any disturbances in the energy balance of tubules might increase 5'-AMP levels in the interstitial

Fig. 9 Myofibroblast in the cortical peritubular interstitium in a rat kidney after 3 days of ureter ligation. **a** The rounded nucleus is surrounded by a large pericaryon, containing enlarged cisterns of rough endoplasmic reticulum (rER); α SMA (arrow) is detectable traversing the cytoplasm; note the abundant fibrillar matrix in the surrounding of the cell. **b** Junction between myofibroblasts processes with stress-like F-actin filaments (asterisk) are frequent; basement membrane-like material is apposed to the plasmalemma (arrows); insert bundle of α SMA filaments with dense bodies (arrows). **c** Inflated rER, filled with flocculent material. Bars **a** 2 μ m; **b, c** and insert 0.5 μ m



space (Le Hir and Kaissling 1993). Thus, hydrolysis of extracellular 5'-AMP by fibroblast 5'NT would elicit local adenosine-mediated responses, like adjustment of blood flow. Under inflammatory conditions also infiltrating activated polymorphonuclear neutrophils may contribute to the pool of interstitial 5'-AMP and adenosine might modulate inflammation (Linden 2006; Yang et al. 2006).

Adenosine adjusts blood supply to organ metabolism by regulating local hemodynamics (Vallon 2003). Thus, the particularly narrow sheathing of glomerular arterioles by 5'NT-positive fibroblasts (Gandhi et al. 1990) (Fig. 10) suggests a role in the regulation of glomerular blood flow. Indeed, studies on 5'NT-deficient mice have confirmed that adenosine mediates the vascular response elicited by changes in NaCl concentration at the macula densa, thereby serving as an important regulator of glomerular filtration rate (Castrop et al. 2004). Due to the narrow apposition of thin 5'NT-positive fibroblast processes to the thin capillary endothelia fibroblasts are often misinterpreted as endothelial cells in immunofluorescence (Vallon 2003) or they are designated as “pericytes”. The soluble guanylyl cyclase (sGC), which is part of the NO-sGC-cGMP signalling pathway associated with regulation of regional hemodynamics (Theilig et al. 2001), and a *b*-type cytochrome 558 as part of the neutrophil NADPH-oxidase (Bachmann and Ramasubbu 1997) have been localized in the cortical fibroblasts.

Extracellular adenosine has been widely implicated in adaptive responses to hypoxia (Eltzschig et al. 2003; Grenz et al. 2007). The role of the fibroblasts in the adaptive response to systemic hypoxia is further highlighted by the discovery that renal erythropoietin is synthesized exclusively by 5'NT-positive renal fibroblasts (Bachmann et al. 1993; Maxwell et al. 1993; Fisher et al. 1996). The kidneys adjust their erythropoietin production to changes in oxygen supply (Maxwell 2003). Regulation of erythropoietin production and transcription of erythropoietin are mediated by hypoxia-inducible factor (HIF Hif-2 α) (Maxwell 2003) which has been located as well to 5'NT-positive fibroblasts (Rosenberger et al. 2003, 2005).

In kidneys of normoxic rats and mice erythropoietin-mRNA is detected only in the 5'NT-positive peritubular fibroblasts in the deep cortex. At this level 5'NT expression by peritubular fibroblasts is highest and it decreases towards the renal surface. Both, hypoxia and anemia, involve a progressive recruitment of additional interstitial fibroblasts for erythropoietin production in a pattern that spreads from the deep cortex toward the capsule (Eckardt et al. 1993). In parallel the 5'NT activity by the peritubular fibroblasts increases towards the capsule (Kaissling et al. 1993). This points again to an obvious link between 5'NT expression (or adenosine) and erythropoietin synthesis,

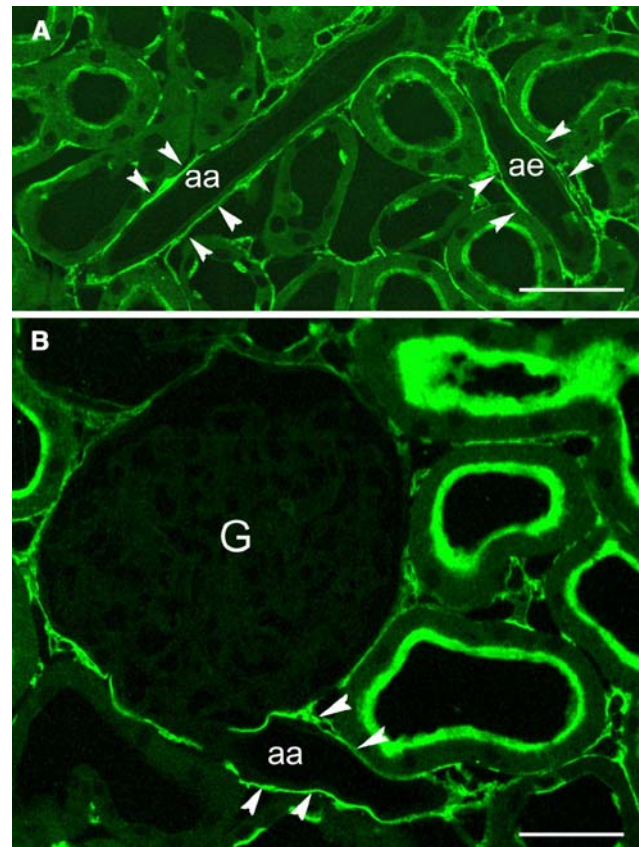


Fig. 10 Glomerular arterioles are ensheathed by ecto-5'-nucleotidase-expressing processes (arrowheads); 5'NT (green); aa afferent arteriole, ae efferent arteriole, G Glomerulus. Bars a 50 μ m; b 20 μ m

which also has been confirmed in a fibroblast cell line (Plotkin and Goligorsky 2006). The details of this link are, however, still inexplicit. Interestingly, Ito-cells (or fat-storing cells) in the liver, which express 5'NT (Schmid et al. 1994) and which resemble morphologically in all respects to the renal cortical fibroblasts, also produce erythropoietin (Maxwell et al. 1994).

The phenotype of fibroblasts changes in acutely (1 week) (Kaissling et al. 1993) and chronically anemic rats (Kurtz et al. 1989). The cortical fibroblasts increase in size, reveal strikingly larger nuclei and frequent lipid droplets in the enlarged pericyon and in peripheral cell processes. In addition to a few sharply outlined large cell processes they display an increased amount of thin, extensively ramified very long processes (Eckardt et al. 1993; Kaissling et al. 1993). The erythropoietin-producing cells show a much higher 5'NT activity in their plasma membrane (Fig. 11) and are consistently negative for α SMA (Maxwell et al. 1993; Plotkin and Goligorsky 2006). Under chronic anemia (3 months) the plasma membrane surface of the 5'NT-positive fibroblasts increases by formation of additional cell processes (Fig. 11) (Le Hir et al. 1989, 1991).

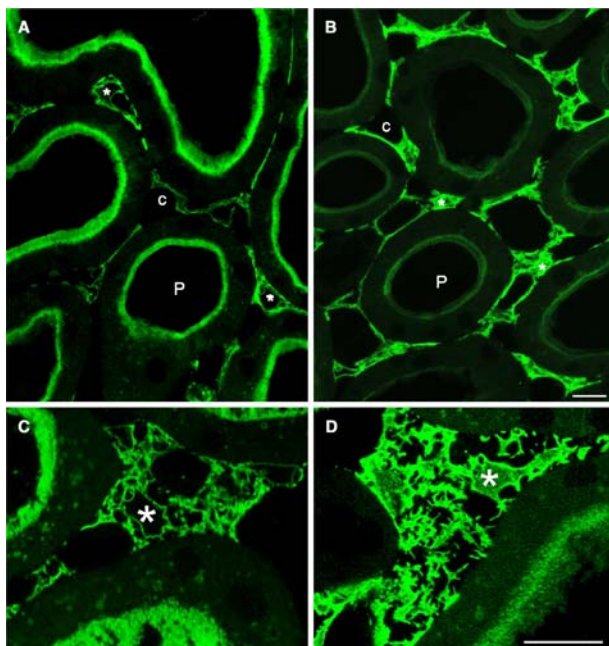


Fig. 11 Ecto-5'-nucleotidase in the renal cortex of a normemic (**a, c**) and an anemic (**b, d**) rat (1 μm cryostat sections; *P* proximal tubules; *c* capillaries). Fibroblasts (*stars*) in anemic rats reveal a markedly increased 5'NT expression and more cellular processes. Bars 10 μm

Role of fibroblasts in chronic renal failure

Fibrosis is the morphological hallmark of chronic renal failure. It is characterized by an exaggerated accumulation of fibrous collagens, by an increased incidence of interstitial cells in the renal interstitium and by the emergence of myofibroblasts.

Origin of fibroblasts/myofibroblasts in renal fibrosis

Until the late 1990s it was generally assumed that myofibroblasts developed by proliferation and differentiation from resident fibroblasts (Bulger and Nagle 1973). Yet, this concept has been challenged in more recent studies. The difficulty to detect interstitial fibroblasts in the kidney, discussed above, resulted in the erroneous belief that fibroblasts are sparse in the healthy kidney. Therefore, sources other than the resident fibroblasts population were proposed for the numerous myofibroblasts found in various models of kidney disease. Three origins have been considered.

Epithelial-mesenchymal transition (EMT)

The hypothesis that in renal diseases, fibroblasts are generated via epithelial-mesenchymal transition (EMT) grounds on two observations. First, in many instances tubular cells lose some of their epithelial features and acquire a “mesen-

chymal” appearance. Second, in such instances some tubular cells express proteins, which are considered specific for fibroblasts/myofibroblasts, namely fibroblasts-specific protein-1 (FSP1) (Zeisberg et al. 2001; Iwano et al. 2002), heat shock protein HSP47 (Iwano et al. 2002) and αSMA (Liu 2004). However the interpretation of the latter findings requires some caution. FSP1 is not a reliable fibroblast marker (Le Hir et al. 2005). HSP47 is supposed fibroblast-specific because it is a chaperone for collagens. However it is not a chaperone for fibrous collagens only. Accordingly, accumulation of HSP47 was observed in tubular cells, which produced excessive amounts of collagen type IV in a model of partial nephrectomy (Kimura et al. 2005). The occasional expression of αSMA in some tubular cells in models or renal disease is intriguing. However, it remains to be proven that αSMA -positive tubular cells acquire the most characteristic function of fibroblasts/myofibroblasts, namely the production of collagen fibers.

In some experimental models, like 5/6 nephrectomy (Kimura et al. 2005), DCT lesion with thiazide diuretics (Le Hir et al. 2005) and UUO (Picard et al. 2008), accumulation of αSMA -positive interstitial cells was observed whereas the tubules remained αSMA -negative. The same observation was made in biopsies of patients with nephritic syndrome (Kuusniemi et al. 2005). At least in those instances myofibroblasts did not appear to originate via EMT.

Bone marrow (BM) origin

There is evidence from animal models and from studies with BM transplant recipients that circulating precursors from BM origin give rise to fibroblasts/myofibroblasts in various organs in adults (Quan et al. 2004; Desmouliere et al. 2005; Hinz et al. 2007). Studies using transgenic rodents have shown a BM origin of a limited fraction of renal myofibroblasts after ischemia/reperfusion injury (Broekema et al. 2007) and in the model of adriamycin-induced chronic renal failure (Li et al. 2007).

Proliferation and differentiation of resident fibroblasts

It is widely accepted that under conditions leading to fibrosis in various organs fibroblasts transform into myofibroblasts, and both fibroblasts and myofibroblasts proliferate (Desmouliere et al. 2005; Hinz 2007; Hinz et al. 2007). Apparently, that straightforward view of the cellular basis of fibrosis has not much attracted the interest of investigators in the field of renal pathophysiology. Thus, very few studies have analyzed the response of resident fibroblasts to renal diseases.

In the following we will discuss a few morphological investigations, which reveal a gradual transformation of

fibroblasts to myofibroblasts. In the experimental model of unilateral ureter obstruction we investigated the phenotype of fibroblasts during the first 4 days after ureter ligation in the rat. Already on day 1 the 5'NT-positive fibroblasts showed de novo expression of α SMA, this increased progressively during the experiment (Figs. 12, 13). The expression of 5'NT decreased at the same time. Furthermore, we observed a massive increase in the mitotic rate in interstitial cells. Mitoses were present in fibroblasts (Fig. 14), myofibroblasts and mononuclear cells. The changes in fibroblast morphology were accompanied by enlargement of the intercellular space and deposition of collagen I (Picard et al. 2008).

In the thiazide-induced model of distal tubular lesion (Loffing et al. 1996) the time course of events resulting in the peritubular inflammation and appearance of myofibroblasts can be particularly well followed. Accumulations of dendritic cells around injured tubular profiles (Fig. 15) were observed after 24 h and massive invasion of mononuclear cells started after about 36 h as well as the morphological changes in peritubular fibroblasts (Fig. 15). Within

three days transformation of fibroblasts into myofibroblasts was indicated by de-novo expression of α SMA and vimentin, an increased complexity of cell shape, an expansion of the RER and an increased incidence of intercellular junctions (Figs. 15, 16) (Le Hir et al. 2005). In contrast to UUO, only fibroblasts in the vicinity of DCTs were affected after thiazide treatment (Fig. 17). That process was interpreted as a transformation of resident fibroblasts in myofibroblasts. Mitotic figures were frequent in fibroblasts and myofibroblasts (Le Hir et al. 2005).

Maxwell et al. (1997) previously observed an expression of α SMA in 5'NT-positive fibroblasts in UUO and after mechanical lesions (needlestick) in mice kidneys, which was associated with, first, a decrease in 5'NT, and second, a reduced capacity of the fibroblasts to respond with erythropoietin production under hypoxia. Proliferation of interstitial cells has also been described in the model of UUO (Duymelinck et al. 2000) and in another model of renal fibrosis induced by aristolochic acid (Pozdzik et al. 2008).

Mechanical stress seems to play a central role in the transformation of fibroblasts into myofibroblasts after ura-

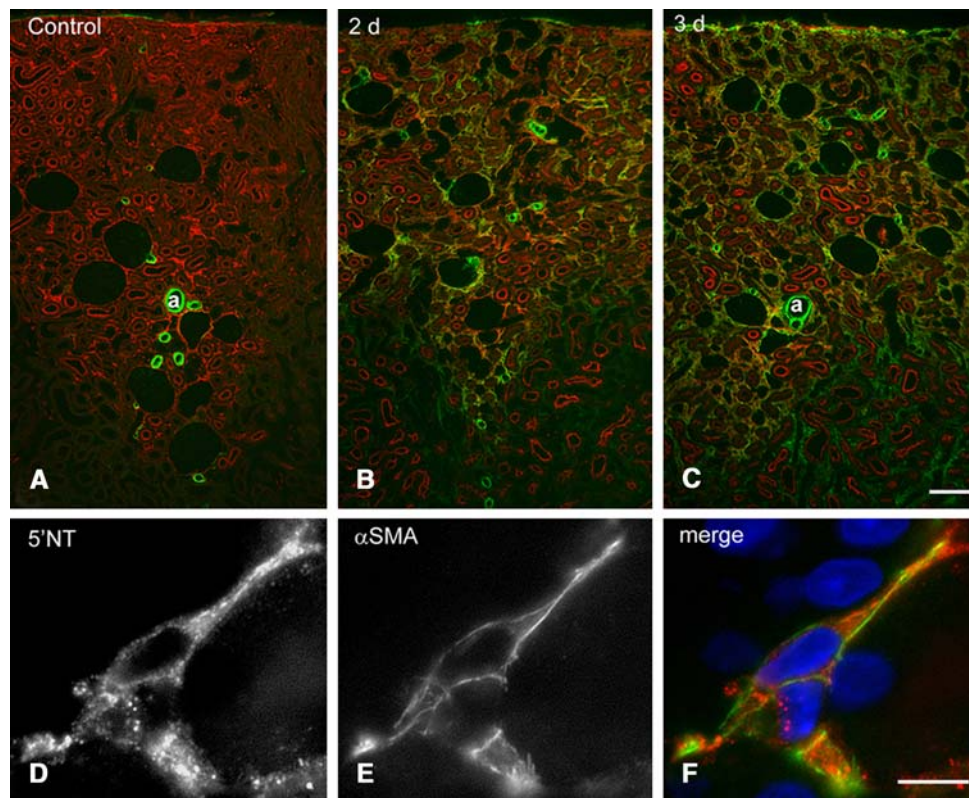
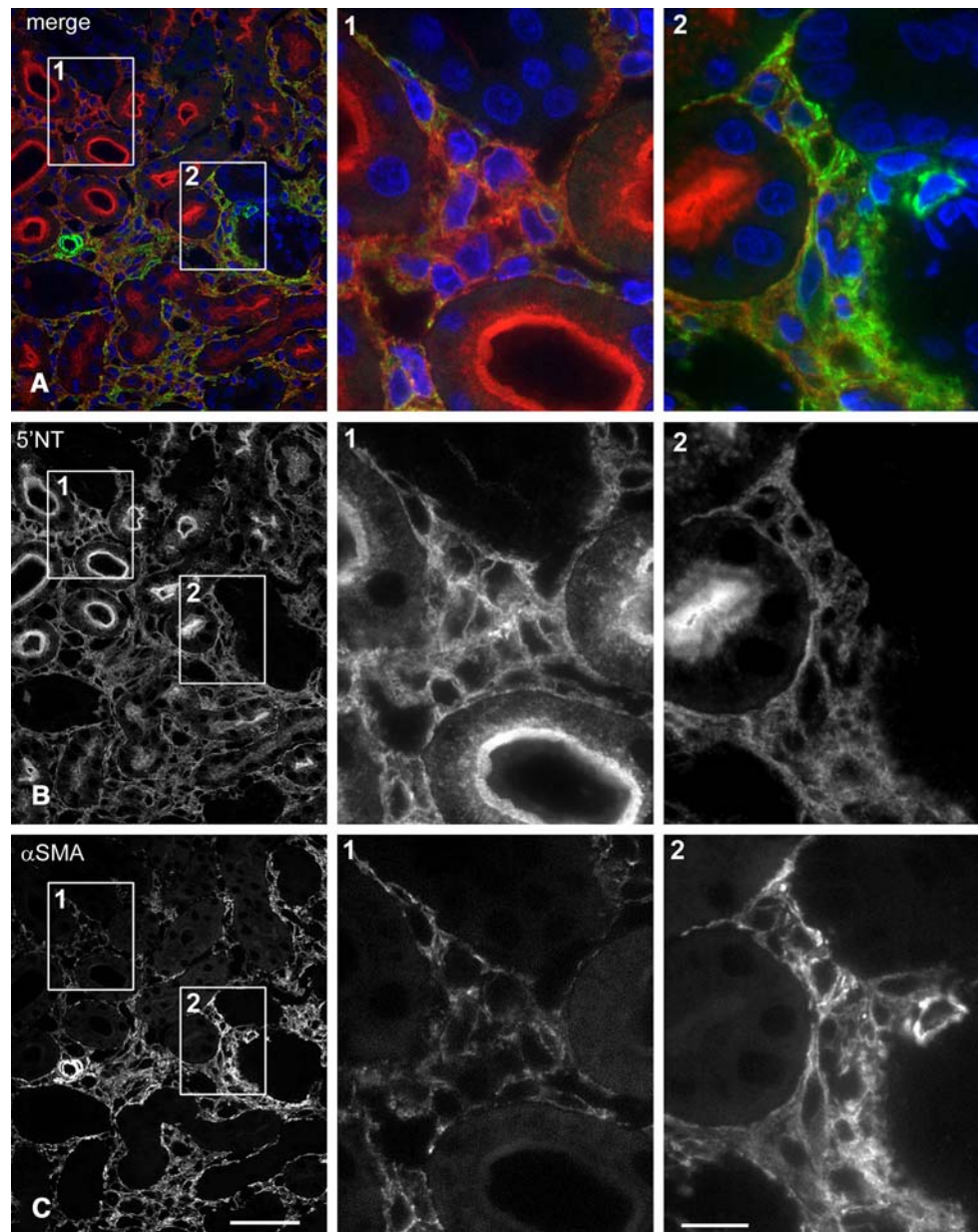


Fig. 12 Renal cortex in sham-operated (a) and in ureter-ligated kidneys (b, c) (3 μ m cryostat sections, red ecto-5' nucleotidase (5'NT), green alpha smooth muscle actin (α SMA), blue cell nuclei). In controls (a) the interstitium and the brush border of proximal tubules are strongly labeled by 5'NT, α SMA labels exclusively arterial vessels (a). After 2 days of ureter ligation (b) interstitial 5'NT staining decreases, whereas α SMA appears and becomes increasingly prominent throughout the

cortex after 3 days (c). Interstitial α SMA-staining progressively appears also in the outer stripe of the outer medulla, seen at the bottom of the images. d–f Interstitial fibroblast in ureter-ligated kidney after 2 days. The weakly expressed 5'NT is distributed in a granular manner over the plasma membrane and the cytoplasm, α SMA is apparent along the plasma membrane and in the cellular processes. Bar a–c 100 μ m, d–f 10 μ m (from Picard et al. 2008)

Fig. 13 Renal cortex after 4 days of ureter ligation [3 μm cryostat sections; *red* ecto-5' nucleotidase (5'NT), *green* α -smooth muscle actin (α SMA), *blue* nuclei]. **a** Merge of channels, **(b)** 5'NT, **(c)** α SMA; the framed area (1) comprises interstitium with still high 5'NT and faint α SMA staining, adjacent to rather intact tubules showing open lumina, the framed area (2) comprises interstitium with strong α SMA and faint 5'NT staining, adjacent to collapsed tubules, showing signs of tubular atrophy. Bar **a–c** $\sim 100 \mu\text{m}$; 1, 2 $\sim 10 \mu\text{m}$ (from Picard et al. 2008)



nyl acetate-induced tubular lesion (Fujigaki et al. 2005). The dilatation or shrinkage of tubules likely exerts a tension on the fibroblasts that are directly attached to damaged tubules. Mechanical forces could be transmitted to further fibroblasts by cell-cell contacts.

Fibrosis and nephron loss: which comes first?

The often massive extent of fibrosis in chronic renal failure unavoidably suggests that fibrosis might be responsible for the destruction of nephrons. Accordingly, in the case of glomerular diseases, the most common cause of chronic renal failure, it has been proposed that proteinuria elicits fibrosis via the release of profibrotic factors by tubular cells. Tubular degeneration would then be a consequence of

fibrosis (Remuzzi and Bertani 1998; Meyer 2003; Eddy 2005). That view is widely accepted but not unchallenged. Indeed, morphological observations suggest that tubular degeneration may be a direct consequence of the encroachment of the glomerular lesion on the tubule (Kriz et al.

Fig. 16 Focal peritubular inflammation, associated with injured distal tubules (D) in rats thiazide-treated for 72 h. **a** 1 μm Epon section; accumulation of interstitial cells around a DCT profile with degenerating epithelial cells, the downstream connecting tubule (CNT) segment and the surrounding interstitium is intact; **b–d** 1 μm cryostat sections; **(b)** dendritic cells (MHC class II, *green*) and 5'NT-labeled fibroblasts (*red*) adjacent injured distal tubule; **(c)** vimentin (*green*) in mononuclear cells (*plus*) and in 5'NT-positive (*red*) fibroblasts (*arrows*); weak expression of vimentin also in the degenerating distal tubular epithelial cells; **(d)** some fibroblasts (*arrows*) show reduced 5'NT (*red*); MHC II-labeled dendritic cells (*green*). Bar 10 μm

Fig. 14 Mitotic cells in the peritubular interstitium in rat kidneys after 1 day (a) and 2 days (b) of ureter-ligation (3 μ m cryostat sections; green α SMA, red 5'NT, blue nuclei). The mitotic cell in a heavily expresses 5'NT and faintly α SMA, the adjacent cell shows strong α SMA and faint 5'NT staining. The 2 mitotic cells in b display strong staining for α SMA along the plasma membrane and in the cell processes, 5'NT staining is weak, granular and is seen over the cytoplasm. Bar 10 μ m (from Picard et al. 2008)

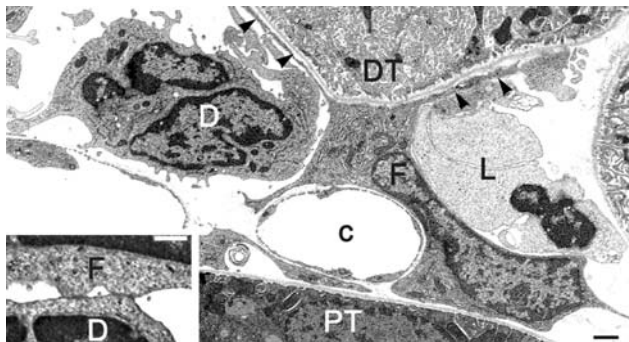
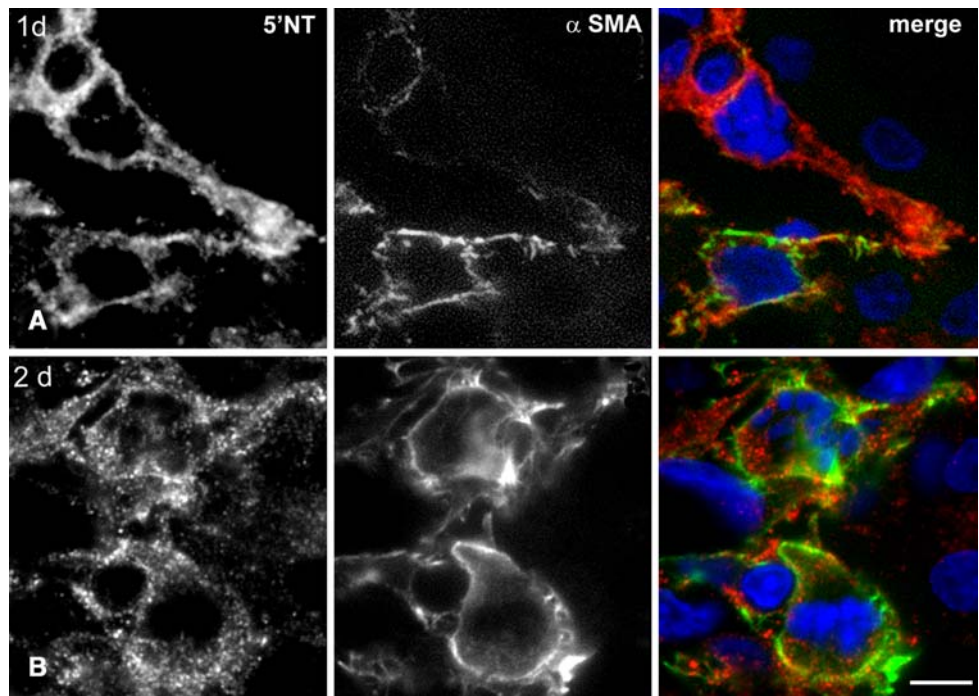
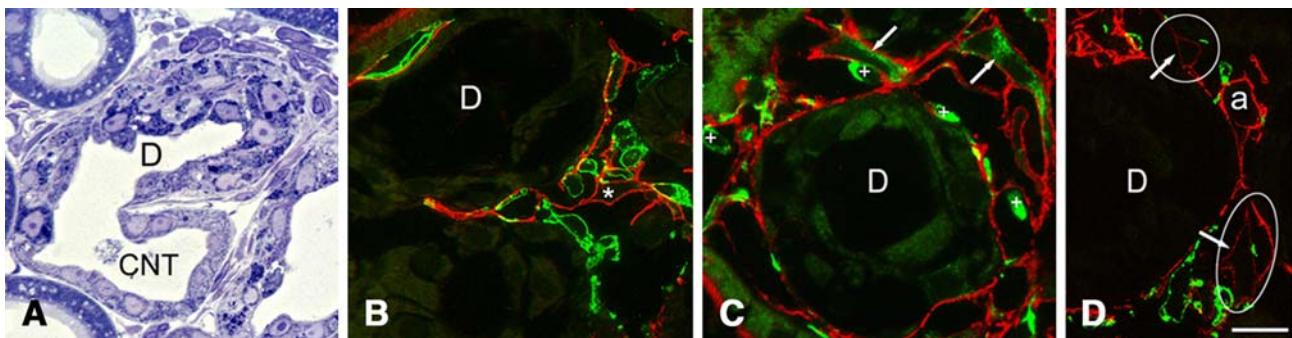


Fig. 15 Cortical peritubular fibroblast in a focal inflammation, related to injury of a distal tubule (Transmission electron microscopy). This figure exemplifies the central position of the fibroblast (F), spanned between a proximal tubule (PT) and an injured cell of a distal convoluted tubule (DT), and its juxtaposition to a capillary (c), a lymphocyte (L) and a dendritic cell (D); two thin processes (arrow heads) of the fibroblast seem to embrace the DT. Signals from the different cells may converge in the fibroblast and elicit an integrated response. Insert “kiss”-like adhesions between a fibroblast and a lymphocyte. Bars 2 μ m, insert 0.5 μ m

2001, 2003; Bertani et al. 2002; Le Hir and Besse-Eschmann 2003; Kriz and LeHir 2005).

In the vast majority of chronic renal diseases there is a mix of tubular, glomerular and vascular alterations mostly accompanied by interstitial inflammation and fibrosis. Obviously, in such situations, it is difficult to decide whether the activation and transformation of fibroblasts is the cause or the consequence of tubular lesions. Therefore it is particularly interesting that in a few models fibrosis appears to be induced by a primary tubular insult. As described above the lesion induced by thiazide diuretics in the DCT in the rat induces an inflammatory reaction and a transformation of fibroblasts into myofibroblasts, which are strictly associated with the injured DCT (Loffing et al. 1996; Le Hir et al. 2005). In the kidney only DCT cells express the transporter NCC, which is the binding site for thiazide diuretics. In this model the fibrotic process came to a halt when under continued metolazone application the NCC-expressing cells were eliminated by apoptosis.



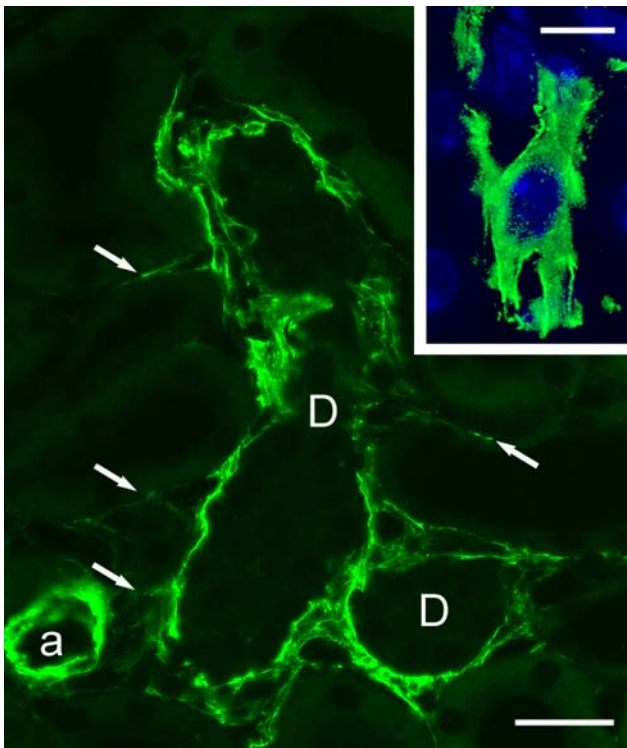


Fig. 17 Up-regulation of alpha smooth muscle actin in the interstitium surrounding injured distal tubular profiles (*D*) of a rat treated for 72 h with a thiazide-diuretic (5 μ m cryostat section). Fibroblasts in extensive contact with the injured tubule show the strongest fluorescence for α SMA. More distant fibroblasts, which appear/are connected to the area of injury by cellular processes (*arrows*), show a weaker upregulation of α SMA; *a* arteriole. *Insert* close view of an interstitial α SMA-positive fibroblast. *Bars* 20 μ m; *insert* 10 μ m

A similar specific association of fibrotic processes with injured tubules was found in other models, in which there is little doubt that the experimental protocol affects primarily tubular cells. Local interstitial fibrosis was observed mainly around distal tubules after treatment with lithium (Zhu et al. 1996). Substances, which are toxic to specific segments of the proximal tubules, induced fibrosis specifically around the affected segments (Fujigaki et al. 2005; Pozdzik et al. 2008).

In those models of primary tubular lesion the association in localization and in time between tubular damages and interstitial responses suggests that fibrosis might represent a repair process. Interestingly, inhibition of transformation of fibroblasts into myofibroblasts after induction of acute renal failure with uranyl acetate resulted in aggravation of renal dysfunction and inhibition of renal repair (Fujigaki et al. 2005).

Conclusion

The cortical fibroblasts contribute essentially to renal functions, not only as skeleton of the organ but also by produc-

ing regulatory substances such as, e.g. adenosine and erythropoietin. In response to tubular lesions fibroblasts proliferate and transform into myofibroblasts, thus initiating fibrosis. The role of that process in renal diseases remains to be elucidated. On one hand, fibrosis might contribute to the deterioration of renal function. On other hand, in the same way as in other organs, fibrosis in the kidney might contribute to repair by containing inflammation and by preserving the organ architecture. Experimental models in which fibrosis is induced by lesions to specific tubular cell types appear particularly promising for the study of the cross-talk between tubular cells and interstitial cells in disease.

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