Role of Atrophic Tubules in Development of Interstitial Fibrosis in Microembolism-Induced Renal Failure in Rat

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We explored the origin and participation of atrophic tubules in the progression of interstitial fibrosis using a new microembolic rat model of chronic renal failure in which foci of atrophic tubules with cufflike basement membrane thickening developed at 4 weeks. Atrophic tubules, immunoreactive for vimentin and platelet-derived growth factor, were surrounded by transformed interstitial cells expressing platelet-derived growth factor receptor β and α -smooth muscle actin. Some tubules in the deep cortex and the outer stripe of the outer medulla had a mosaic appearance. Tall, intact proximal tubular cells with a brush border and positivity for *Phaseolus vulgaris* erythroagglutinin, adjoined typical atrophic tubule cells having no brush border and an immunostaining pattern characteristic for atrophic tubules. The transformed interstitial cells expressing α -smooth muscle actin were located near atrophic but not intact tubular epithelial cells. Type IV collagen accumulated between damaged tubular cells and transformed interstitial cells. Heat shock protein 47 showed immunoreactivity in damaged epithelial cells and in interstitial myofibroblasts. Staining with an anti-endothelial antibody suggested damage to peritubular capillaries near atrophic tubules. By disturbance of microcirculation following microsphere injection, proximal tubular cells expressed vimentin and platelet-derived growth factor; diffusion of the latter presumably stimulated transformation of interstitial cells to myofibroblasts. Injured tubular epithelial cells and interstitial myofibroblasts both were responsible for interstitial fibrosis. (*Am J Pathol* 2001, 158:75–85)

renal diseases.^{5–7} Though many investigators have studied the mechanism of progression of interstitial damage in chronic renal disease, the details of pathogenesis remain to be clarified.^{8–10}

Atrophy and the dilation of tubules, and interstitial fibrosis have been assessed together as tubulointerstitial changes and considered *en bloc* as evidence of end-stage kidney disease.^{9,11} However, which of these changes in the tubules and interstitium are responsible for the progression of chronic renal disease and also how the specific changes interrelate causally is not clear. Furthermore, the origin and fate of these atrophic and dilated tubules are uncertain. El Nahas has suggested that tubular atrophy results from functional overload or increased metabolism in hypertrophic (dilated) tubules, although this hypothesis is not supported by conclusive evidence.¹¹ Other previous studies have stressed the importance of damaged tubules in promoting tubulointerstitial injury, since tubular cells in a damaged kidney can express or secrete various cytokines including growth factors^{12,13} and matrix proteins.^{14,15} In addition, a trans-differentiation of tubular epithelial cells into myofibroblasts¹⁶ and high proliferation index among atrophic tubular cells have been noted in end-stage human kidneys with interstitial fibrosis.¹⁷ Since pathological changes in tubules occur at the same time as interstitial fibrosis in most animal models of renal disease, causal relationships between tubular changes and interstitial fibrosis have been difficult to delineate.

Recently we established a nonimmunologic model of progressive renal failure induced by microembolism in rats. In this model, relatively undamaged tubules are mingled with damaged tubules beginning in initial stages of renal failure,¹⁸ thus resembling human chronic renal diseases. The characteristic histological feature of this model is development of atrophic tubules in advance of any notable glomerular lesions, massive proteinuria, or hypertension. The appearance of atrophic tubules also precedes development of dilated tubules. These findings suggest that atrophic tubules may somehow incite progression of renal disease.

Tubulointerstitial changes, as opposed to glomerular scarring, are known to reflect deterioration of renal function.^{1–4} Thus the extent and severity of interstitial lesions are considered key factors in progression of chronic

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In this study, we used the new model to investigate the origin of atrophic tubules and their involvement in accumulation of myofibroblasts and matrix protein deposition in immediately surrounding interstitial tissues. We identified "mosaic tubules" that included both normal tubular epithelial cells and damaged cells, presenting valuable clues to the origin and pathogenetic role of atrophic tubules.

Materials and Methods

Animal Model

Male Wistar rats 12 weeks of age weighing 270 to 300 g were obtained from SLC (Hamamatsu, Japan), and were allowed free access to standard laboratory chow and water. Microembolism was produced as described in a previous report.¹⁸ In brief, the right kidney was removed using sodium pentobarbital (40 mg/kg, i.p.) for anesthesia. Microspheres (acryl beads 20 to 30 μ m in diameter, approximately 5×10^5 per rat, kindly provided by Dr. Takabayashi, Hamamatsu College, University of Shizuoka, Japan) were suspended in 0.5 ml of normal saline and injected slowly into the aorta through a 27-gauge needle placed immediately caudal to the ostium of the left renal artery. During microsphere injection, the aorta caudal to the site of needle insertion as well as the anterior mesenteric and celiac arteries were clamped to direct the microspheres into the left renal artery. Blood flow through the left renal artery was maintained throughout this procedure. In control rats, normal saline instead of the microsphere suspension was injected following right nephrectomy.

Light Microscopic Examination

Five rats each were killed in saline- and microsphere-injected groups before and 4, 8, and 12 weeks after injection. The left kidneys were removed after perfusion with 10 ml of cold saline and then fixed in methyl Carnoy's solution. Paraffin-embedded sections 2 μ m thick were stained using periodic acid-Schiff (PAS) or Masson's trichrome method.

Histochemical and Immunohistochemical Examinations

For histochemical and immunohistochemical examinations, 4- μ m sections were prepared and stained by an avidin-biotin-horseradish peroxidase method (Histofine SAB-PO kit; Nitierei, Tokyo, Japan). To determine the origin of atrophic tubules, the following antibodies or markers were used: biotin-labeled lectin from *Phaseolus vulgaris* erythroagglutinin (PHA-E; Sigma, St. Louis, MO) for the proximal tubule¹⁷; sheep polyclonal antibody against human Tamm-Horsfall glycoprotein (THP; Chemicon International, Temecula, CA) for the thick ascending limb of the loop of Henle¹⁹; biotin-labeled peanut agglutinin (PNA; Biomed, Foster City, CA) for the distal tubule¹⁹;

and cytokeratin (DAKO, Glostrup, Denmark) for connecting and collecting tubules.²⁰ It is well known that some antibodies to cytokeratin can stain all components of tubules in human kidneys and their stainability for tubular segments is quite different between antibodies. The antibody we used stained only connecting and collecting tubules in rat kidney in a preliminary study. To investigate the mechanism of tubular basement membrane thickening and fibrosis of the surrounding interstitium, several antigens were examined in tubular epithelial cells and interstitium using antibodies as follows: mouse monoclonal antibodies against platelet-derived growth factor (PDGF) (monoclonal antibody to PDGF-B chain, PGF-007, a gift from Mochida Pharmaceutical, Tokyo, Japan); human α -smooth muscle actin (α -SMA, DAKO); vimentin (from porcine ocular lens, Sigma); rabbit polyclonal antibodies against PDGF receptor β (PDGF-R, Santa Cruz Biotechnology, Santa Cruz, CA); and type IV collagen (LSL, Tokyo, Japan). The secondary antibodies were biotin-labeled donkey sera against mouse, rabbit, or sheep IgG (Chemicon International). Collagen-synthesizing cells were identified by staining heat shock protein (HSP) 47 with a mouse monoclonal antibody (Stressgen; Victoria, BC, Canada). This HSP is recognized as a collagen-specific molecular chaperone that plays a pivotal role in the biosynthesis, processing, and secretion of procollagen from endoplasmic reticulum.²¹⁻²³ The number and the integrity of peritubular capillaries were assessed using an endothelial cell-specific mouse monoclonal antibody to rat RECA-1 antigen (Serotec, Sapporo, Japan).

Morphometric Study

To evaluate the interrelationship among antigen expression in tubulointerstitial cells (PDGF, PDGF-R, and α -SMA) and histological change (frequency of atrophic tubules and severity of fibrosis), morphometric studies were performed in 15 rats (5 rats each at 4 weeks, 8 weeks, and 12 weeks after surgery). Numbers of atrophic tubules and PDGF-positive tubules were counted in 10 fields at a magnification of $\times 100$. Atrophic tubules were identified immunohistochemically using anti-vimentin antibody, since most cells appearing atrophic or damaged were seen to express vimentin in their cytoplasm. PDGF-R- or α -SMA-positive interstitial cells were counted in 10 fields at a magnification of $\times 200$. The number of positive tubules or cells in each field was summed for use in comparisons. The extent of interstitial fibrosis was determined in Masson's trichrome-stained sections by point-counting method using an eyepiece with a 10×10 grid. Under a magnification of $\times 100$, green-stained areas overlapping grid-crossing points in 20 fields were summed and expressed as a percentage of the total area.

To assess the change of the peritubular capillary blood flow in the interstitial lesions, the number of capillaries and the size of their lumens in the normal and injured area were separately measured in sections stained by anti-RECA-1 antibody from 5 rats killed at 8 weeks. Rats killed 8 weeks after microsphere injection were selected because the injured areas were moderately scattered in the

renal interstitium. An injured area was defined by existence of atrophic and mosaic tubules and interstitial fibrosis. The areas of the normal and injured area were measured by point-counting method. The numbers of peritubular capillaries were counted in 5 fields in each section under a magnification of $\times 200$ and expressed as the density per $1 \mu\text{m}^2$. The luminal area of the peritubular capillaries was measured with an image analyzer (Macscope, Mitani corp., Maruoka-cho, Japan), tracing the internal surface of 50 peritubular capillaries each in the normal and injured area and the mean value was calculated for statistical analysis.

Statistical Analysis

Correlation coefficients were determined using linear regression analysis. Statistical analysis was performed using Student's *t*-test. Differences were considered significant for $P < 0.05$.

Results

Microspheres were found mainly in afferent arterioles and in glomerular capillary lumens near the vascular pole. Most glomeruli that had trapped a microsphere in a capillary lumen showed nearly normal architecture in the capillary tufts. Some glomeruli, often in clusters, demonstrated collapse of capillary tufts that probably reflected ischemia due to the afferent arteriole obstruction. Totally sclerosed glomeruli, however, were found only rarely.

Atrophic tubules with thickened basement membranes were scattered among normal tubules, mainly in the deep cortex and in the outer stripe of the outer medulla, and could be seen as early as 4 weeks after microsphere injection (Figure 1, A and B); with increasing intervals, the number of atrophic tubules steadily increased (4 weeks, $4.04 \pm 2.26/10$ fields; 8 weeks, $6.26 \pm 3.92/10$ fields; 12 weeks, $36.0 \pm 5.45/10$ fields). Many dilated tubules without thickening of tubular basement membrane were evident at 8 weeks after injection (Figure 1C), after which numbers of dilated tubules increased gradually. By 12 weeks after injection, markedly dilated tubules were numerous and sometimes contained large casts. Interstitial fibrosis was prominent.

Cells of atrophic tubules did not have a brush border and lacked immunostaining for markers of specific tubule segments including PHA-E, THP, PNA, and cytokeratin (not illustrated). The epithelial cells of atrophic tubules all strongly expressed vimentin and PDGF (Figure 2, B and C). Many interstitial cells surrounding these atrophic tubules specifically expressed PDGF-R (Figure 2D). A significant correlation was found between numbers of PDGF-positive tubules and PDGF-R-positive interstitial cells (Figure 3A). About one-third of the PDGF-R-positive cells concomitantly expressed α -SMA (Figures 2E and 3B). Numbers of PDGF-R-positive and α -SMA-positive cells in the interstitium were slightly increased at 4 weeks following microsphere injection, showing rapid increase in parallel with increasing histological damage. A significant correlation was found between the numbers of

PDGF-positive tubules and α -SMA-positive cells (Figure 3C). The degree of interstitial fibrosis was significantly related both to numbers of atrophic tubules and to α -SMA-positive cells (Figure 3, D and E). In control animals without embolization, PDGF was expressed only in distal tubules. PDGF-R-positive fibroblasts were sparsely scattered in the interstitium, and no α -SMA-positive cells were found (not shown).

By 4 weeks after microsphere injection, some tubules showed a peculiar appearance, being composed of two kinds of cells; we named these "mosaic tubules" (Figure 4, A and B). One group of cells were tall, had a brush border, and expressed PHA-E, all characteristic of proximal tubular cells. These cells did not express vimentin or PDGF. The other cells in the mosaic were pale and low, lacking a brush border; they strongly expressed vimentin and PDGF, but not PHA-E (Figure 4, C–E). The basement membrane underlying damaged tubular epithelial cells was multilayered and thickened, while normal-appearing tubular epithelial cells in mosaic tubules were situated on a thin basement membrane that appeared to be normal (Figure 4, A and B). Interstitial cells staining for α -SMA or PDGF-R were found near the mosaic tubules, but cells positive for both α -SMA and PDGF-R were almost exclusively in the vicinity of vimentin- and PDGF-positive tubular cells (Figure 4, F and G). Few myofibroblasts were found in contact with normal proximal tubular cells showing a brush border. These mosaic tubules were found most frequently in the deep cortex and probably in part in the outer stripe of the outer medulla (Figure 5). They were intermingled with the atrophic tubules, and often found on the fringe of the atrophic tubule clusters.

Anti-HSP 47 antibody strongly stained both vimentin-positive atrophic tubule cells and α -SMA-positive interstitial cells (Figure 6, A–C). Type IV collagen showed accumulation between them (Figure 6D). Other areas distant from atrophic tubules showed only sparse distribution of collagen, especially in early stages of injury.

To assess the damage to peritubular capillaries, we stained endothelial cells with a monoclonal antibody against the rat RECA-1 antigen. Normal tubules were surrounded by two to three large patent capillaries, while capillaries near atrophic and mosaic tubules showed decreases in size, and the RECA-1 staining intensity (Figure 7). The semiquantitative analysis of luminal area of peritubular capillaries were significantly decreased (Figure 8). No significant change in the density of capillaries was observed (Figures 7 and 8).

Discussion

In this study we examined immunohistochemically evident changes in rats with microembolism-induced chronic renal failure, where proteinuria develops after the microembolism and is followed by a decrease in renal function. Damaged and undamaged tubules closely co-exist in this model as in human renal diseases. Since tubule damage occurs before development of notable morphological changes in glomeruli, massive proteinuria, or hypertension, this model of chronic renal failure is well

Figure 1. Light micrographs of rat kidney after microsphere injection (**A** and **C**, Masson's trichrome stain; **B**, PAS stain). In **A**, atrophic tubules with thickened basement membranes are seen among apparently normal tubules 4 weeks after microsphere injection ($\times 100$). In **B**, also at 4 weeks, atrophic tubule cells rested on a thickened, multilayered basement membrane. These cells have pale, flattened cell bodies and no brush borders ($\times 600$). In **C**, at 8 weeks, many dilated tubules without basement membrane thickening are seen ($\times 100$).

suited to determining how tubule damage produces chronic renal failure.¹⁸

Tubulointerstitial changes in chronic renal diseases include atrophic tubules, dilated tubules, cast formation, cellular infiltration, and interstitial fibrosis. In our microembolic model, tubule atrophy preceded dilation of tubules, cast formation, or interstitial fibrosis. Thus, the model also is appropriate for study of the mechanisms underlying development of atrophic tubules and relationships between tubular atrophy and interstitial fibrosis. In a standard model of chronic renal failure, 5/6 nephrectomized rats, individual components of tubulointerstitial lesions, such as atrophic tubules, dilated tubules, and interstitial fibrosis develop almost simultaneously, interfering with evaluation of the effects and interrelationships of the components (unpublished observations).

The present study aimed to elucidate the pathogenesis of atrophy in tubules and of surrounding matrix protein accumulation in the early stage of our microembolization model. First, the origin of atrophic tubules was determined by immunostaining for several multiple site-specific tubular antigens (PHA-E reactive molecules for proximal tubules, THP for thick ascending limbs, PNA reactive T-antigen for distal tubules, and cytokeratin for connecting and collecting tubules). None of these proteins were expressed in atrophic tubules, though loss of previously existing immunoreactivity may have occurred in the course of atrophy. In this context, our finding of mosaic tubules is instructive: cells with brush borders were positive for PHA-E, identifying them proximal tubule epithelial cells while other, typically atrophic cells lacked a brush border and immunoreactivity for PHA-E, rested on a lamellated, thickened basement membrane and were immunoreactive for vimentin and PDGF. The mosaic tubules indicated that the atrophic tubules originally were proximal tubules.

Atrophic tubules were found as early as 4 weeks after microsphere injection. Development of atrophic tubules preceded such glomerular alterations as hyalinosis, necrosis, and tuft adhesion, which are thought to be induced by hyperfiltration. In addition, atrophic tubules were observed before development of severe proteinuria, hypertension, or hypercholesterolemia.¹⁸ These findings suggested that the atrophic changes in tubules resulted directly from microembolism rather than secondary effects via hypertension, hypercholesterolemia, or glomerular injury.

Using an immunohistochemical marker for capillaries, we found that near atrophic and mosaic tubules the size of capillaries were significantly decreased. The partially atrophic mosaic tubules were observed mainly in the deep cortex and probably in the outer stripe of the outer medulla, regions known to be particularly vulnerable to ischemic stress.^{24,25} These findings suggested that the changes in atrophic tubules and mosaic tubules were caused by disturbed microcirculation, though we cannot exclude a possibility that the decreased capillary area near atrophic tubules was caused by the lack of reabsorptive function of atrophic tubules. In this regard, several investigations about the relationship between atrophic tubules and disturbance of microcirculation have

Figure 2. Masson's trichrome staining (**A**) and immunohistochemical staining (**B–E**) of atrophic tubules in serial renal sections from a rat killed 4 weeks after microsphere injection (all $\times 200$). In **A**, atrophic tubules are surrounded by a broad zone of matrix protein, most likely collagen fibers. In contrast, normal tubules have thin basement membranes. In **B**, atrophic tubule cells strongly express vimentin in their cytoplasm (**arrow**), and some interstitial cells also are stained (**arrowhead**). Intact proximal tubules do not express vimentin, as in the upper portion of this field. In **C**, PDGF-B chain is stained only in atrophic tubules (lower portion). Staining elsewhere is comparable to that in unembolized negative controls. In **D**, PDGF-R is strongly positive in tissues surrounding atrophic tubules. Sparse and faint staining of cells also can be found near intact proximal tubules. Expression of PDGF-R in the upper part might indicate fibroblasts in patchy fibrous areas, which are also found in the normal kidney. Those PDGF-R-positive interstitial cells have no relation to adjacent expression of vimentin (**B**), PDGF (**C**), or α -SMA (**E**). In **E**, α -SMA is stained in cells surrounding atrophic tubules. Cells staining appear to represent a portion of cells expressing PDGF-R.

been reported so far.^{26–29} Møller et al reported that, as the chronic renal disease progressed, the distance between tubules and adjacent peritubular capillaries increased and the fraction of tubular circumference facing capillaries decreased.²⁶ Similar explanation for an adverse effect of interstitial fibrosis on renal function was described by Morrissey and Klahr²⁷ in the review on NF- κ B regulation of renal fibrosis. Ohashi et al²⁸ demonstrated a significant decline in the number of the peritubular capillaries, followed by tubulointerstitial scarring in a rat experimental glomerulonephritis. In addition, Thomas et al²⁹ suggested a possibility that the tubulointerstitial injury in aging rats is the consequence of ischemia secondary to peritubular capillary injury and altered eNOS expression.

In experimentally induced anemia, Kaissling et al³⁰ showed that the proximal tubules display structural changes which seem to be correlated to hypoxia and a volume of the peritubular space was increased. These effects were evident only in the cortical labyrinth. Their

findings are instructive to analyze the results in this study. However, the hypoxic stress in anemic condition and in the current model might not be the same. For example, anemia is usually associated with the increase in blood flow. In contrast the renal blood flow is probably decreased in the current model. In addition, anemia might induce other physiological adaptations, systemic and local, in the host. Another difference is that their experiment was a short term one, only for 8 days, in comparison to the present long-term, 12-week, experiment. These differences might be the cause of the differences in the cites showing pathological changes.

We also found small but significant increase in proteinuria developing 4 weeks after microsphere injection.¹⁸ This suggested that hyperfiltration of remained glomeruli had already started. Although we think that it is unlikely that hyperfiltration alone induced the histological tubulointerstitial change, we cannot exclude an effect of hyperfiltration on the appearance of atrophic tubules. Hypoxic stress on tubular cells might be determined by the bal-

Figure 3. Correlations among numbers of tubules expressing PDGF, interstitial cells expressing PDGF-R, interstitial cells expressing α -SMA, atrophic tubules, and area involved by interstitial fibrosis. **A:** PDGF-positive tubules versus PDGF-R-positive interstitial cells; $r = 0.843$, $P < 0.01$. **B:** PDGF-R-positive interstitial cells versus α -SMA-positive interstitial cells; $r = 0.912$, $P < 0.01$. **C:** PDGF-positive tubules versus α -SMA-positive interstitial cells; $r = 0.881$, $P < 0.01$. **D:** Number of atrophic tubules versus area of interstitial fibrosis; $r = 0.826$, $P < 0.01$. **E:** α -SMA-positive interstitial cells versus area of interstitial fibrosis; $r = 0.853$, $P < 0.01$.

ance of oxygen demand and supply, not only absolute value of blood flow. It is likely that nephrons with hyperfiltration would be more susceptible to suffer from hypoxia. To distinguish exactly the contributions of hypoxia and hyperfiltration, further studies should be done.

The pale epithelial cytoplasm of atrophic tubules strongly expressed vimentin, which is not expressed by normal tubular epithelial cells but is known to be ex-

pressed by degenerating and regenerating tubular cells.³¹⁻³³ In this model, sloughing and regeneration of proximal tubular cells, such as observed in acute renal failure, did not occur. Therefore, we assumed that vimentin-positive tubular epithelial cells were damaged ones. However, as we reported in the previous paper, proliferating cell nuclear antigen (PCNA) was expressed in some cells of atrophic tubules.¹⁸ This suggested that some of

Figure 4. Mixed expression of antigens among epithelial cells of a mosaic tubules (★) examined in serial sections (×400). In **A**, collagen fibers occupy a restricted area near damaged, vimentin-positive, PDGF-positive tubular epithelial cells (Masson's trichrome). In **B**, the basement membrane underlying damaged tubular epithelial cells is lamellated and thickened (arrow). In **C**, a proximal tubule marker, molecules adhering to PHA-E, are demonstrated in intact proximal tubules and in intact epithelial cells in the mosaic tubule, especially those with brush borders. Damaged epithelial cells do not express these molecules. In **D**, damaged epithelial cells express vimentin in their cytoplasm (arrow), whereas intact epithelial cells of proximal and distal tubules do not. In **E**, immunohistochemical staining for the PDGF-B chain is seen in the damaged proximal epithelial cells, also positive for vimentin (arrow). A distal tubule (△) shows positive staining. In **F**, PDGF-R is expressed chiefly in interstitial cells adjacent to damaged tubular epithelial cells (arrow). In **G**, staining of α-SMA is seen in interstitial cells adjacent to damaged epithelial cells (arrow).

Figure 5. Distribution of mosaic tubules, stained with anti-vimentin antibody 8 weeks after microsphere injection ($\times 30$). Mosaic tubules marked by **asterisks** are distributed mainly in the deep cortex. A part of them seems to be located in the outer stripe of the outer medulla according to the relationship to the inner stripe.

cells of atrophic tubules might be regenerating cells. In this regard, Hall and colleagues^{34,35} reported that PCNA is involved in unscheduled DNA synthesis, i.e., nucleotide excision-repair process, and the expression of PCNA is a necessary but not sufficient requirement for proliferation; accordingly the PCNA-positive, vimentin-positive atrophic tubular cells also might be damaged ones.

PDGF was co-expressed with vimentin in these atrophic proximal tubular epithelial cells. The distal tubule constitutively expressed PDGF, although staining was weak³⁶ and did not show enhancement by embolization in this model even in advanced stages. Renal expression of PDGF has been localized to platelets, monocytes/macrophages, glomerular mesangial and epithelial cells, epithelial cells of inner medullary collecting ducts, and renal fibroblasts.³⁷ Kliem et al³⁶ reported that in 5/6 nephrectomized rats, PDGF-B chain expression was increased in distal tubules and collecting ducts, and was also weakly positive in some proximal tubules, particularly in areas of tubulointerstitial injury. Prominent proximal tubular expression of PDGF, which we found in our model, has not been reported in the past. The cause of these differences is not known, but may reflect differences in stimulation for PDGF expression or difficulty in

identifying proximal tubules with degenerative changes in previous studies. In our microembolic model, stimulation for PDGF expression on proximal tubules such as hypoxic stress may be more intense than in other models.^{38–41}

To investigate the presumed target for PDGF produced in injured tubules, we examined the expression of PDGF-R, which showed strong staining in interstitial cells, most likely fibroblasts,^{37,42} around the atrophic tubules. In control animals and in the intact interstitium of diseased rats, a small number of PDGF-R-positive interstitial cells were found in patchy fibrous areas, especially around vessels. However, the epithelial cells in atrophic tubules did not express PDGF-R. These results agree with Kliem et al,³⁶ who reported that atrophic tubules with flattened epithelium were surrounded by strongly PDGF-R-positive interstitial cells in 5/6 nephrectomized rats.

PDGF has been reported to have chemotactic and mitogenic effects on renal interstitial fibroblasts and to transform these cells into myofibroblasts.^{36,37,43} Tang et al⁴³ demonstrated that administration of the PDGF-BB dimer at a dose of 5 mg/kg induced tubulointerstitial cell proliferation and fibrosis. In that study, expression of α -SMA indicated that transformation to myofibroblast be-

Figure 6. Immunohistochemical staining of an atrophic tubule with antibodies against HSP 47 (**A**), vimentin (**B**), α -SMA (**C**), and type IV collagen (**D**). Vimentin-positive proximal tubular epithelial cells and α -SMA-positive myofibroblasts both express HSP 47. In a zone between these cell types, a massive accumulation of type IV collagen is demonstrated. Normal proximal epithelial cells do not express HSP 47 ($\times 200$).

Figure 7. Immunohistochemical staining for vimentin (**A**) and RECA-1 (**B**) in serial sections. RECA-1 expression was attenuated near vimentin-positive, atrophic tubules (**arrows**) in comparison to strong staining near intact tubules (**arrowheads**). Magnification, $\times 90$.

Figure 8. The luminal area (A) and the density (B) of peritubular capillaries in the normal and injured areas in kidneys 8 weeks after microsphere injection. Mean $\pm$ SD.

gan on day 3 and peaked at day 5 and declined markedly by day 21. These authors concluded that PDGF-BB might be an important mediator of tubulointerstitial hyperplasia and fibrosis. In the current study α -SMA expression closely correlated with PDGF-R expression, and about one-third of PDGF-R-positive cells also expressed α -SMA. Numbers of cells immunostaining for α -SMA increased in parallel with interstitial fibrosis. Surrounding the atrophic tubules, matrix protein staining green by Masson's trichrome method accumulated circumferentially, and excluded capillaries from this peritubular zone. In mosaic tubules, myofibroblasts and peritubular matrix protein accumulation were spatially restricted to the area adjoining vimentin- and PDGF-positive cells. Few myofibroblasts and little matrix protein were found in contact with normal proximal tubular cells that possessed a brush border. On the basis of these findings, we assumed that PDGF released from damaged proximal tubules activated interstitial fibroblasts via PDGF-R present on the fibroblasts, and transform them to myofibroblasts. These interactions between damaged proximal tubules and myofibroblasts stimulated production of abundant collagen fibers to result in interstitial fibrosis. The precise role of PDGF in this model, however, has not been fully determined, and its elucidation will require further studies.

Fibroblasts, myofibroblasts, proximal tubular cells, and macrophages all have been proposed as a candidate for extracellular matrix protein-synthesizing cells in renal interstitial fibrosis.^{44–48} When we examined expression of HSP 47, which is known to increase in parallel with collagen production,^{21–23} interstitial myofibroblasts and damaged proximal tubular cells both were positive, which suggested that both interstitial cells and tubular cells may synthesize abundant collagen fibers that form a fibrotic zone between atrophic tubules and myofibroblasts. However, because HSP 47 is an indirect evidence for collagen synthesis, we have to confirm collagen synthesis in those cells by other direct means such as *in situ* hybridization before bringing a final conclusion.

In conclusion, we propose the following pathogenetic scheme concerning atrophic tubules and surrounding fibrosis in our model (Figure 9). Following multiple microembolism, a disturbance in the interstitial microcirculation causes functional damage in a portion of the epithelial cells of proximal tubules, resulting in atrophic tubules.

Figure 9. A proposed pathogenesis for the development of fibrosis surrounding atrophic tubules.

The atrophic cells, which express vimentin, PDGF, and HSP 47, secrete PDGF into the interstitial space, which promotes myofibroblastic transformation of interstitial fibroblasts through PDGF-R. The myofibroblasts synthesize collagen fibers, which are deposited in a zone between these cells and atrophic tubular cells. In addition, atrophic tubular cells may themselves produce collagen fibers, thus playing a dual role as a stimulator and an effector of fibrosis. Deposition of matrix protein, in turn, causes a disturbance in the adjacent microcirculation that injures neighboring epithelial cells. Once established, this sequence acts as a vicious circle of progressive tubulointerstitial injury.

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