Human Renal Cortical Interstitial Cells With Some Features of Smooth Muscle Cells Participate in Tubulointerstitial and Crescentic Glomerular Injury[1]

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ABSTRACT

In most forms of renal injury, even those due to a primary glomerular process, the extent of tubulointerstitial scarring is a critical determinant of renal functional reserve and prognosis. Yet, little is known about the functional characteristics of the interstitial cells that mediate the processes of chronic tubulointerstitial injury. In this study, tissues from normal kidney ($N = 7$), from nephrectomies removed for allograft rejection ($N = 14$) and chronic pyelonephritis ($N = 2$), and from a cohort of 128 biopsies exhibiting a range of glomerulopathies and tubulointerstitial injury were characterized with antibodies to mesenchymal cells (α-smooth muscle actin, desmin) by immunohistology. Selected normal kidneys were also studied by immunoelectron microscopy. Normal adult kidneys contain a widespread population of cortical interstitial cells that constitutively express α-smooth muscle actin but not desmin. Immunoelectron microscopy shows that these cells are fibroblasts and not capillary endothelial cells or leukocytes. It has previously been shown that these cells constitutively express platelet-derived growth factor receptor β and the p75 nerve growth factor receptor. Accumulations of cells expressing smooth muscle actin were identified at sites of chronic tubulointerstitial injury in allograft and pyelonephritic kidneys. The cohort of 128 renal biopsies also revealed accumulations of muscle actin–expressing cells at sites of interstitial injury. These findings demonstrate that a population of interstitial cells with some muscle-like features can be identified in normal kidneys. These cells apparently can proliferate at and/or migrate to sites of tubulointerstitial injury. The constitutive expression of nerve growth factor receptor and platelet-derived growth factor receptor β by these cells suggests potential signaling mechanisms involving corresponding growth factors that may recruit or activate these cells at sites of injury.

Key Words: *Interstitium, fibroblasts, fibrosis, actin, cytoskeleton, crescentic glomerulonephritis*

The importance of chronic tubulointerstitial injury as the critical factor in determining the degree of fixed impairment of renal function in patients with renal disease has been increasingly accepted over the past decade (1–7). However, despite our ability to readily identify lesions of tubular atrophy and interstitial fibrosis in human renal biopsies, little is known about the human cortical interstitial cell, its characteristic phenotype, and the mechanisms by which such cells are stimulated to engage in activities that result in lesions of chronic tubulointerstitial injury.

In this study, we used immunolocalization techniques at the light microscopic and ultrastructural levels for the cytoskeletal protein α-smooth muscle actin to identify a widespread population of interstitial cells in normal adult kidneys with some phenotypic features common to smooth muscle cells. We have previously provided evidence that these cells also constitutively express the platelet derived growth factor receptor β subunit (PDGFR) (8) and the p75 nerve growth factor receptor (NGFR) (9). Furthermore, by studying human renal biopsies exhibiting a variety of glomerulopathies and a wide range of tubulointerstitial injury, we identified a prominent accumulation of α-smooth muscle actin-bearing cells at sites of interstitial injury. These cells are also found around glomeruli containing organizing crescents, suggesting possible cell migration from the interstitium into damaged glomeruli. We hypothesize that these cells are derived from a resting population of smooth muscle actin$^+$, desmin$^-$, PDGFR$^+$, and NGFR$^+$ interstitial cells normally present in mature human kidneys.

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MATERIALS AND METHODS

Source of Tissue

Normal human kidney tissue ($N = 9$) was obtained from kidneys surgically excised because of the presence of a localized neoplasm. Tissues used for this study were obtained from macroscopically normal portions of kidney located at some distance from the neoplastic process. Additional normal tissue was obtained from fresh cadaver kidneys unable to be used for transplantation ($N = 3$).

Diseased human kidney was studied from a consecutive series of renal biopsies obtained over a 2-yr period (1983 to 1984 at the University of Washington), as previously detailed (10). Biopsies were excluded from this study for the following reasons: (*1*) tissue submitted in inappropriate fixative; (*2*) insufficient tissue remaining after the completion of a diagnostic workup; (*3*) fewer than three glomeruli remaining in tissue sections available for immunohistochemical studies. One hundred twenty-one cases with acceptable tissue were studied. These cases were obtained from patients with a broad range of renal diseases (Table 1).

TABLE 1. Renal biopsy cases evaluated for actin expression[a]

Diagnosis	No. of Cases
Minimal Change Disease	15
Focal and Segmental Glomerulosclerosis	11
Mesangial Proliferative Glomerulopathy (Nonimmune Complex)	7
Membranous GN (Nonlupus)	3
IgA Nephropathy (Predominantly Sclerosing)	4
IgA Nephropathy (All Other)	9
Membranoproliferative GN	3
Lobular GN	3
Amyloid	8
Diabetic Nephropathy	13
Diffuse Lupus Nephritis	9
Membranous Lupus Nephritis	3
Acute GN	5
Postinfectious	3
Cryoglobulinemic	1
NOC	1
Crescentic GN	14
Transplant Rejection	9
Normals	12

[a] Abbreviations: GN, glomerulonephritis; NOC, not otherwise classifiable.

Fixation

All tissues were fixed in methyl Carnoy's solution (60% methanol, 30% chloroform, 10% acetic acid) and were processed and embedded in paraffin by conventional techniques (11). Normal tissues studied by immunoelectron microscopy were fixed either in 4% paraformaldehyde or periodate-lysine-paraformaldehyde as previously described (8).

Immunohistochemistry

Sections of methyl Carnoy's fixed tissue were deparaffinized with xylene and graded ethanols, blocked with 3% hydrogen peroxide, and washed with phosphate-buffered saline (PBS) (138 mM NaCl, 2.7 mM KCl, 3.2 mM Na_2HPO_4, 1.5 mM KH_2PO_4; pH 7.3). The tissue was then incubated with one of the primary murine monoclonal antibodies (see below) and was subsequently processed by a streptavidin-biotin-immunoperoxidase technique. 3,3'-Diaminobenzidine with nickel chloride enhancement served as the chromogen. Sections were counterstained with methyl green.

Antibodies

Three murine monoclonal antimuscle antibodies were used for immunohistochemical evaluation. Monoclonal antibody 1A4 (DAKO Corp., Carpinteria, CA) (12), with specificity for the smooth muscle α-actin isoform and HHF35 (13), reactive with all four "muscle-specific" actin isoforms (smooth muscle α and γ, cardiac muscle α, and striated muscle α-actin isoforms) has been well characterized previously. A murine monoclonal antibody to human desmin (DAKO Corp.), an intermediate filament demonstrable in some populations of human smooth muscle cells, was also used. For all biopsies, a negative control consisted of the substitution of the primary antibody with both irrelevant murine monoclonal antibodies and PBS. Positive internal controls in each biopsy consisted of actin-positive smooth muscle cells present in the renal vasculature for the 1A4, HHF35, and antidesmin antibodies.

Lectins

Endothelial cells of peritubular capillaries were identified by lectin binding studies with Ulex europaeus I lectin (Vector Laboratories, Burlingame, CA) as previously described (14,15).

Immunoelectron Microscopy

Frozen, 4% paraformaldehyde-fixed kidneys were sectioned at 6 μm, and sections were adhered to aminopropylmethoxysilane-coated slides and air dried for 30 min. The sections were then stained by being hydrated in PBS for 15 min, incubated in 0.05% sodium borohydride in PBS at 4°C for 60 min to

reduce free aldehyde groups, rinsed, and then incubated with antibodies 1A 4 or HHF35 or control antibody at 4 μg/mL in PBS containing 2% BSA overnight at 4°C. The slides were then processed as above by the streptavidin-biotin-immunoperoxidase method. After being washed in distilled water, sections were reacted with 2% OsO_4 for 1 h at room temperature, rinsed, and then dehydrated through graded ethanols and into propylene oxide. Sections were then infiltrated with a 50/50 mixture of PolyBed (PolySciences, Inc, Warrington, PA) and propylene oxide for 1 h. Beem capsules were filled with PolyBed, inverted over the sections, infiltrated overnight, and then polymerized at 55°C for 48 h. The blocks were removed by heating the slide briefly and quickly snapping off the capsule. Thin, 0.1-μm sections were cut, mounted on grids, and examined with a Philips 410 electron microscope.

Alternatively, periodate-lysine-paraformaldehyde-fixed kidneys were embedded in LR white resin (Ted Pella, Inc., Redding, CA) after dehydration through graded ethanols. After polymerization at 55°C for 48 h, the blocks were thin sectioned and the sections were mounted on formvar-coated nickel grids. The sections were then immunostained with HHF35, followed by colloidal gold–conjugated goat anti-mouse antisera (Amersham Corp., Arlington Heights, IL). After brief counterstaining in uranyl acetate, the grids were examined with a Philips 410 electron microscope.

RESULTS

Normal Kidney

In all cases, more than 50% of the cortical interstitial areas examined contained readily identifiable populations of spindle-shaped peritubular cells that were reactive with antibodies to both α-smooth muscle actin and pan-muscle actin (Figure 1A and B), but not to desmin, another protein commonly expressed by smooth muscle cells (see reference 31) (Figure 1C). By low-power microscopic examination, the distribution of these cells between closely packed tubular structures resembles the distribution of the peritubular capillary network, as indicated by the staining of capillaries by the endothelial binding lectin Ulex I (Figure 1D). At high powers of resolution, it can be shown that the spindled cells bearing a smooth-muscle-like phenotype are instead located within the interstitium, in a location characteristic of fibroblasts between the capillary network and tubular basement membranes (Figure 1E). In some cases, usually those with large blocks of tissue within the individual section, there was some variability in the expression of muscle specific actin by interstitial cells within the same tissue section. This appears to be the result of technical reasons, *i.e.*, either due to problems in fixation or a general weakening of antigenicity due to prolonged storage of the specimens used in this study, or due at times to the large tissue sections used, with resultant variability in tissue exposure to fixative, incubating antibodies, or secondary antibody detection systems.

Kidney Biopsy Series

Interstitium. Uninjured portions of renal cortical interstitial tissues were generally indistinguishable from the interstitial patterns of actin and desmin expression described above in normal kidneys. Areas of interstitial fibrosis revealed increased numbers of actin-expressing cells (Figures 1B and 2A to C). There was no up-regulation of desmin expression detectable in these areas. Increased numbers of actin$^+$ cells were also identified in areas of active interstitial nephritis encountered in both transplant rejection biopsies (Figure 2D) and the interstitial nephritis accompanying some glomerulonephridites such as crescentic glomerulonephritis (Figure 2E and F) and chronic pyelonephritis.

Glomeruli. The up-regulation of α-actin expression in glomeruli within this series has been previously documented (10). However, two additional features not emphasized in that study, but relating to this evaluation of actin expression by interstitial cells, are as follows. Areas of periglomerular fibrosis often showed a prominent, concentric accumulation of actin-positive interstitial cells, a feature that regularly characterized this process (Figure 3). In the small set of biopsies demonstrating crescent formation, we also observed the presence of a population of actin-positive cells within crescents, demonstrating features of fibrocellular to fibrous organization (Figure 3C to E). These cells were not encountered in the few examples of acute cellular crescents available for this study (Figure 3B). In some of the organizing crescents, the actin-positive cells identified appeared to form a morphologic continuum with accumulations of similar actin-positive cells present in the interstitial tissue immediately adjacent to the glomerulus (Figure 3C to E). Rarely, it appeared that actin$^+$ interstitial cells derived from the periglomerular cell population were in fact migrating through the glomerular space to participate in the organization of the crescent.

Immunoelectron Microscopy. Ultrastructural immunolabeling for either α-actin or pan-muscle actin, with both immunogold and immunoperoxidase labels, revealed widespread decoration of the cytoplasm of the fibroblast-like interstitial cell population, which was characterized by long cytoplasmic processes, the presence of prominent rough endoplasmic reticulum, and the absence of cell attachment structures (Figure 4). No labeling of peritubular capillary endothelial cells or adjacent tubular epithe-

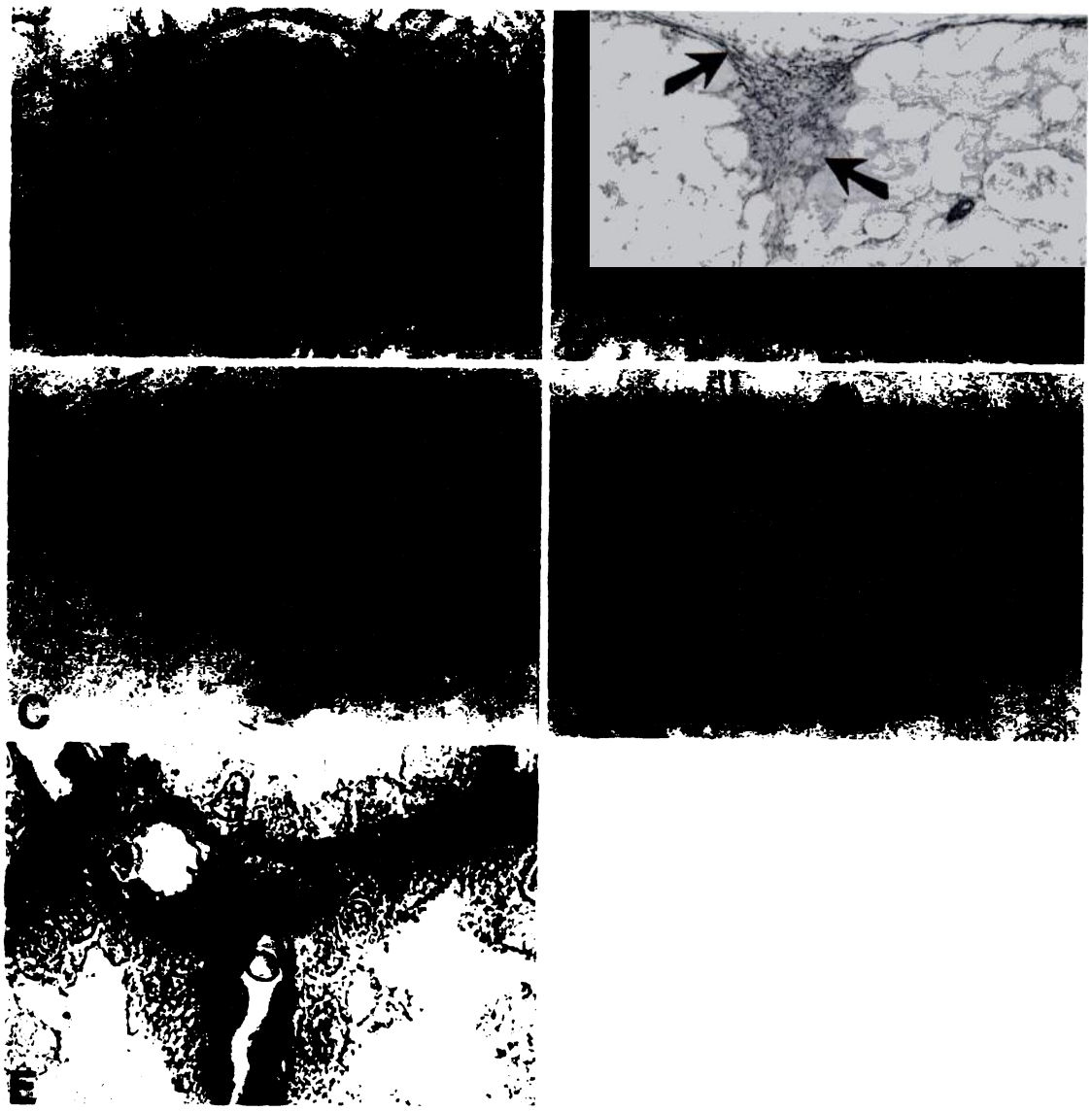

Figure 1. (A) Low-power micrograph of normal parenchyma of a kidney removed for localized carcinoma. There is widespread expression by intertubular cells of α-actin (black reaction product). Other cells demonstrating α-actin expression include some mesangial cells and the smooth muscle cells of an arteriole, seen both in cross-section and longitudinal section extending to bottom of the figure. Original magnification, ×160. (B) Same kidney as in Panel A. A focus of nephrosclerotic change resulting in the focal retraction of the subscapular cortex is accompanied by a localized increase of α-actin-expressing interstitial cells (arrows). Original magnification, ×55. (C) Normal kidney tissue demonstrating expression of desmin by many smooth muscle cells present in muscular arteries. There is no detectable expression of desmin by interstitial cells. Original magnification, ×105. (D) Same kidney as in Panels A and B. Endothelial binding of the lectin Ulex europaeus I delineates glomerular and peritubular capillaries. At low and intermediate powers of magnification, the intertubular pattern of Ulex binding is similar to patterns of α-actin expression. Original magnification, ×190. (E) High magnification of normal kidney demonstrates that the interstitial cells that express α-actin are located between the peritubular capillaries and the tubular basement membranes. Original magnification, ×520. All panels, immunoperoxidase technique, methyl green counterstain.

lial cells was identified. Interstitial leukocytes were only rarely identified in our ultrastructural surveys of the cortical interstitium. No expression of actin was identified in any such cells encountered.

DISCUSSION

α-Smooth muscle actin, one of six actin isoforms identified in mammalian cells, has received consid-

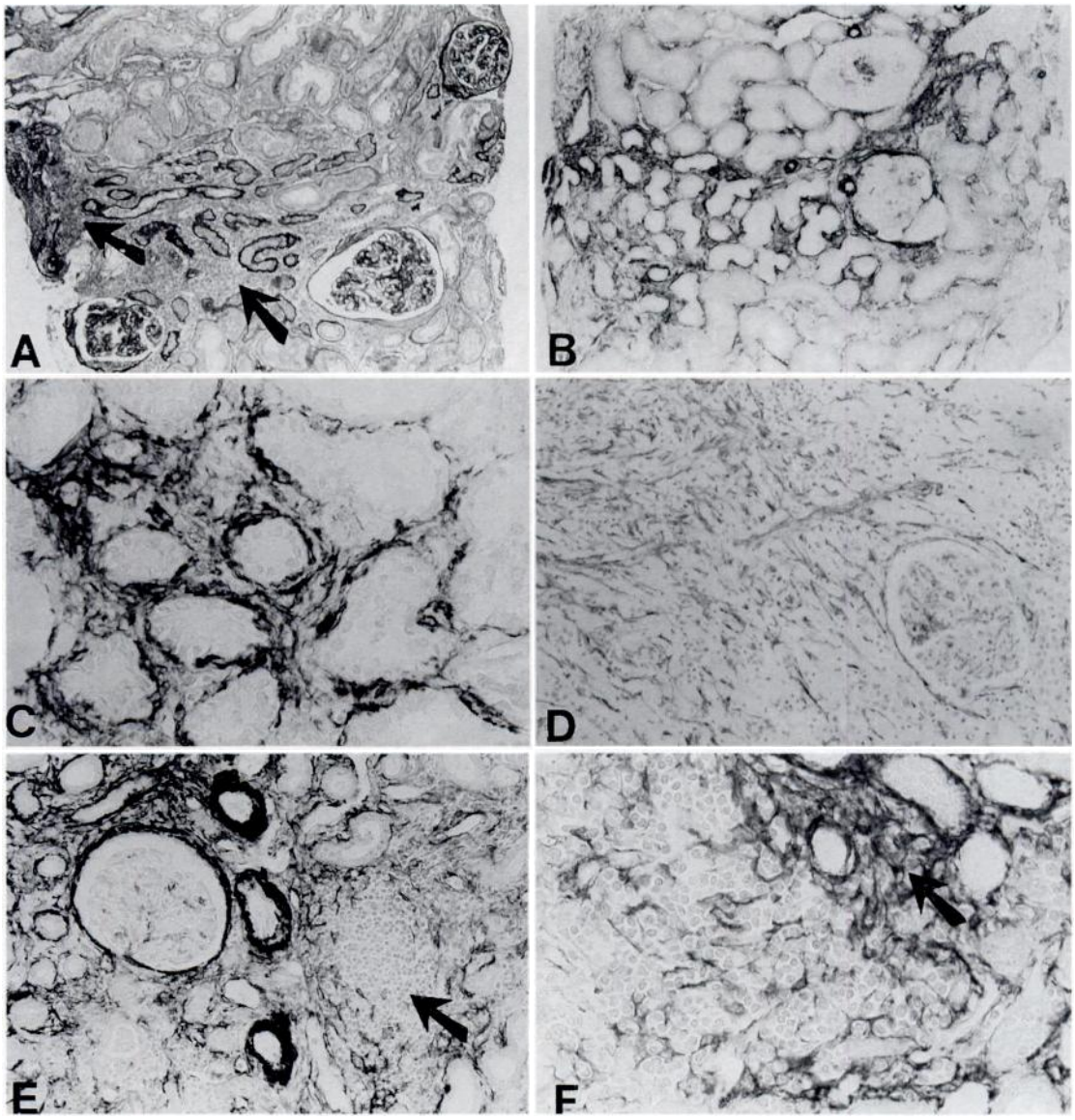

Figure 2. (A) Renal biopsy of a case showing localized zones of tubular atrophy and interstitial fibrosis (arrows), with adjacent areas of well-preserved tubular architecture. Periodic acid–Schiff stain. Original magnification, ×65. (B) Same biopsy as in Panel A. Immunostaining demonstrates increased numbers of actin-expressing interstitial cells in zones of interstitial fibrosis compared with their distribution in normal-appearing adjacent tubular parenchyma. Original magnification, ×65. (C) Interstitium in a renal biopsy in which the principal injury was crescentic glomerulonephritis. Areas of interstitial fibrosis, characterized by widening of the intertubular space and accumulations of matrix material, also typically contain accumulations of interstitial cells expressing α-actin. Original magnification, ×190. (D) Acute cellular (interstitial) rejection in a kidney allograft biopsy. There is a prominent interstitial inflammatory infiltrate composed of lymphocytes and monocytes, but there is also an interspersed population of dark-staining, α-actin-expressing mesenchymal cells. Original magnification, ×105. (E) A case of crescentic glomerulonephritis associated with focally prominent interstitial nephritis. There is α-actin expression by arterial smooth muscle cells, periglomerular cells, and interstitial cells in areas of fibrosis. Areas of most concentrated interstitial inflammation (arrow) show localized diminution and even absence of interstitial α-actin expression. Original magnification, ×90. (F) Same case as in Panel C. Areas of prominent interstitial inflammation (at left) are adjacent to areas of chronic interstitial fibrosing injury (arrow). As in examples of transplant rejection, a population of spindle-shaped, α-actin-expressing cells are interspersed among (migrating into ?) the regions of active inflammatory injury. Original magnification, ×190. Panels B to F, immunoperoxidase technique, methyl green counterstain.

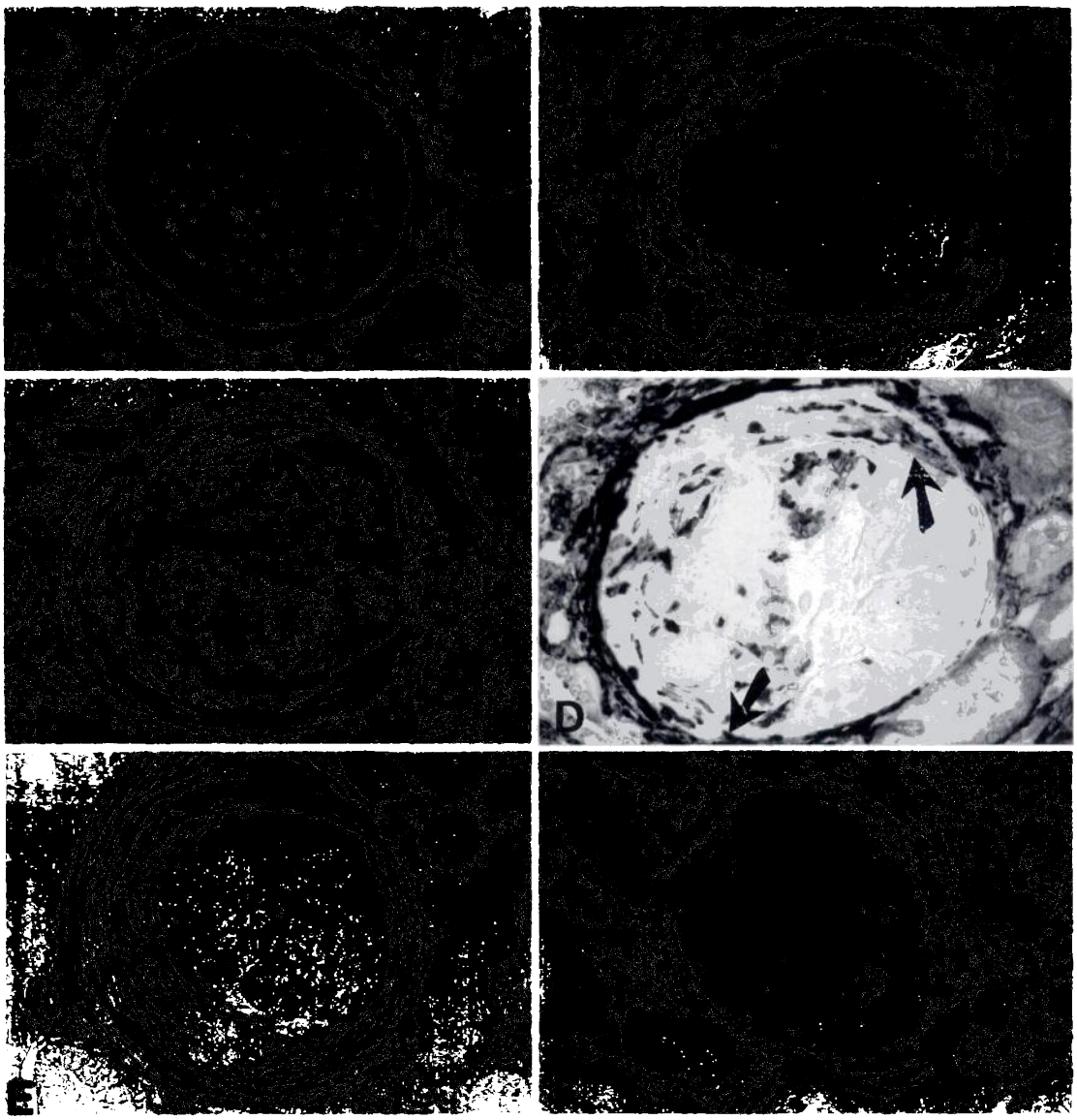

Figure 3. (A) Morphologically early stage of periglomerular fibrosis. Glomerular tuft appears undamaged, but there are multiple concentric layers of α-actin-expressing interstitial cells immediately external to Bowman's capsule. Original magnification, ×250. (B) Cellular crescent formation. There are accumulations of periglomerular and interstitial cells expressing α-actin, but no such cells are identifiable within the crescent. Original magnification, ×250. (C) Early transition from cellular to fibrocellular crescent. α-Actin-expressing cells can be seen migrating into the crescent (arrow). Mesangial cells also focally express α-actin. Original magnification, ×250. (D) Fibrocellular crescent from a case of amyloidosis contains α-actin-expressing spindled cells, perhaps arising from the periglomerular accumulations of such cells (arrows). Some mesangial cells also show prominent α-actin expression. Original magnification, ×335. (E) More advanced organization of fibrocellular crescent, with numerous α-actin-expressing cells present in crescent and periglomerular interstitium. Original magnification, ×250. (F) Crescentic glomerulonephritis. End-stage, globally sclerotic glomerulus from a case of crescentic glomerulonephritis. At this advanced stage of injury, only rare, scattered foci of α-actin proteins typically can be identified. Original magnification, ×250. All panels, immunoperoxidase technique, methyl green counterstain.

erable attention as a specific differentiation marker for cells of smooth muscle origin (12,16–19). α-Smooth muscle actin is normally expressed by smooth muscle cells, myoepithelial cells, (18,19), and vascular pericytes (19,20). The expression of this marker in stromal cells of otherwise uncertain relation to smooth muscle cells has been identified in testis, ovarian thecal cells, reticular cells of lymph nodes, and stromal cells of the intestine (reviewed in Reference 19); this feature has then been used to suggest that such cells are indeed smooth muscle-like in their ontogenetic development. In addition,

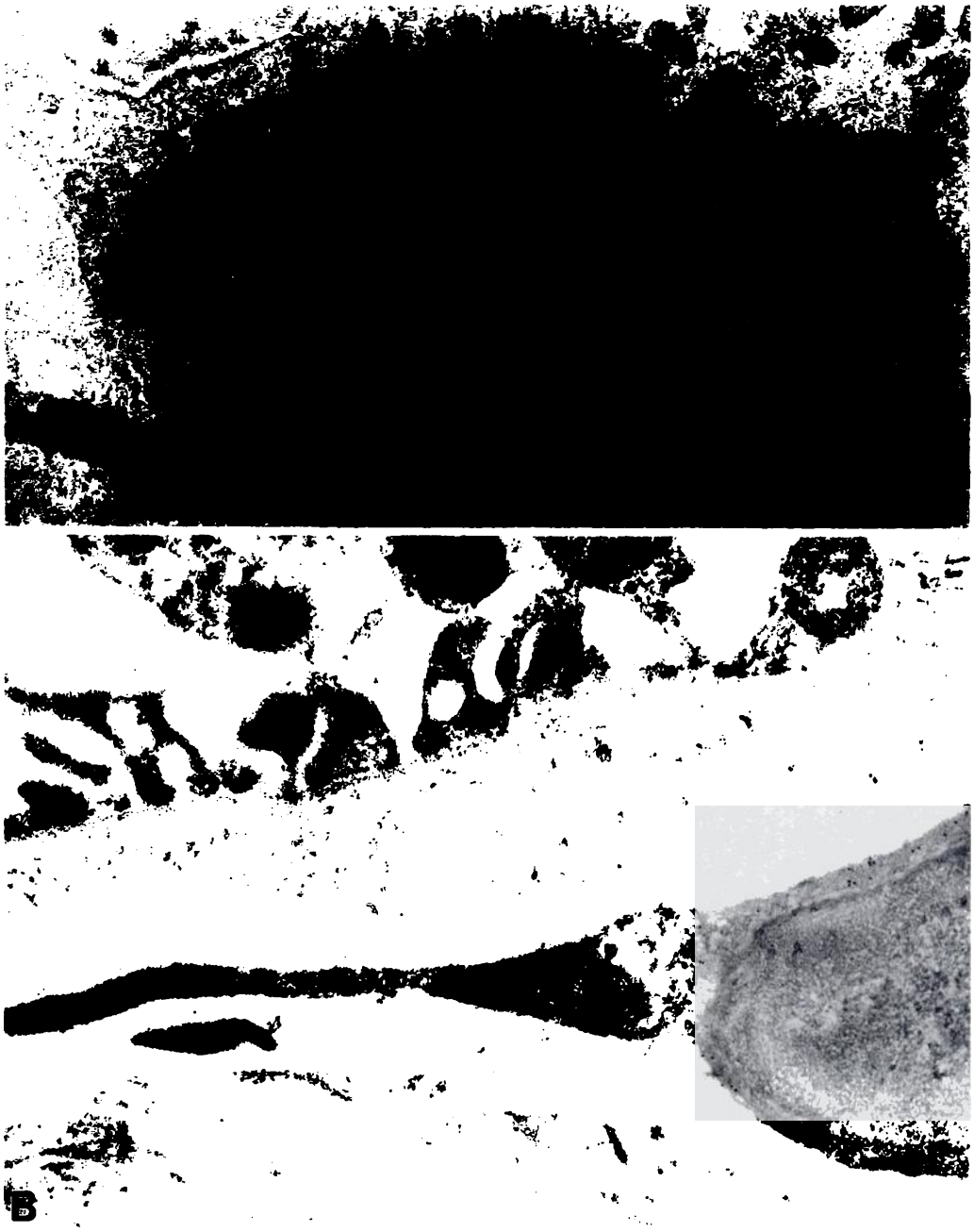

Figure 4. (A) Immunoelectron micrograph demonstrating black peroxidase reaction product in the cytoplasm of an interstitial fibroblast. The peroxidase is conjugated to the antibody to α-smooth muscle actin. The cytoplasm contains abundant rough endoplasmic reticulum. There is no staining of adjacent tubule (T) or tubular basement membrane (B). Original magnification, ×7,900. Immunoperoxidase labeling technique. (B) Immunoelectron micrograph of normal kidney interstitium with an immunogold label conjugated to HHF35, a pan-muscle actin-specific antibody. The gold label is concentrated in the cytoplasm and cellular processes of an interstitial fibroblast, without similar labeling of adjacent interstitial collagen, tubules, or tubular basement membranes. Original magnification, ×30,400. Immunogold labeling technique.

the expression of this marker by mesenchymal cells with features of fibroblasts has been used in part to define the myofibroblast, a cell type intermediate in phenotype between fibroblasts and smooth muscle cells (19,21). These cells have been identified in healing wounds and scarring processes in such organs as skin (22) and lung (23,24) and may also be transiently created from fibroblasts in cell culture by exposure to cytokines or other stimuli likely to be found in an inflammatory process (25). However, because exam-

ples of anomalous, low-level expression of "muscle-specific" isoforms have been observed in nonmuscle-derived cells both *in vivo* and *in vitro* (19,21,26–29), this phenotypic feature alone may only indicate a smooth muscle cell/myofibroblast-like quality of some cell types without necessarily denoting their origin from smooth muscle cells.

This study began as a survey to study actin expression in human glomerular mesangial cells in the setting of glomerulonephritis (10), after our initial observations that this phenotypic alteration was associated with mesangial injury and proliferation in the rodent model of anti-Thy 1. glomerulonephritis (30). An unexpected finding was the widespread, albeit not uniform, expression by cortical interstitial cells of α-smooth muscle actin in apparently undamaged interstitium. This actin isoform was recognized by two distinct, well-characterized monoclonal antibodies reactive with different actin epitopes; one antibody specifically recognizes epitopes restricted to α-smooth muscle actin (12), and one antibody recognizes a less restricted epitope of α-smooth muscle actin that is also present in other "muscle-specific", but not nonmuscle, actin isoforms (13). Both of these antibodies have been characterized by western blotting and by correlative analysis of actin mRNA by northern blotting in rodent models of nephritis, and both have been investigated in a variety of disease processes by the tissue immunocytochemical procedures used in this study (10).

Examination by conventional transmission electron microscopy indicates that the interstitial cells that express α-actin seem to be located adjacent to tubular basement membranes rather than in a pericyte-like location adjacent to the peritubular capillaries (Figure 2). They are indistinguishable from the cortical interstitial cells described in the rabbit kidney by Bulger and Nagle (32) and in rat kidney by Bohman and by Lemley and Kriz (33,34) and conform to the descriptions of human medullary interstitial cells made by Bulger *et al.* (35). These cells are typical of fibroblasts in that they possess abundant rough endoplasmic reticulum and long cytoplasmic processes and are without the cell attachment structures of epithelial cells, the granules and lysosomes typical of neutrophils and phagocytic leukocytes, and the organized filaments and extracellular basement membrane material that characterize smooth muscle cells. Using the high degree of resolution afforded by immunoelectron microscopy techniques, we now demonstrate that it is these peritubular interstitial fibroblast cells, and not peritubular capillary endothelial cells or some other cell type, that are labeled by both gold-conjugated and peroxidase-labeled monoclonal antibodies reactive with muscle-specific actin and α-smooth muscle actin (Figure 4).

In some ways, the demonstration that human renal interstitial cells express actins generally considered to be specific to smooth muscle cells is not surprising. In a study of rabbit kidneys injured by complete ureteral obstruction, Nagle *et al.* observed that interstitial fibroblasts underwent a morphologic transformation to form cells containing numerous actin-like cytoplasmic filaments as detected by transmission electron microscopy; further, these cells expressed antigens that were reactive with antisera with anti-smooth muscle specificities obtained from human patients with hepatitis (36). MacPherson *et al.* also noted interstitial cells reactive with one of the anti-smooth muscle actin antibodies used in this study while evaluating kidneys for actin expression by glomerular cells, although they did not further characterize the involved cell population or investigate the role of such cells in disease (37). Finally, in a model of angiotensin-induced hypertensive interstitial injury, we have demonstrated that the expression of α-smooth muscle actin can be dramatically up-regulated in rat interstitial fibroblasts, which unlike those of humans, do not have baseline immunocytochemically detectable expression of this protein (38). Using techniques of light microscopy, immunocytochemistry, and immunoelectron microscopy similar to those used in this study, we have previously shown that these actin-expressing interstitial cells in human kidneys also express PDGFR-β and p75 NGFR (8,9). Taken together, we believe that these studies begin to define the principal population of cortical interstitial cells in mature human kidney as having some phenotypic features of myofibroblasts, *i.e.*, cells with features intermediate between fibroblasts and smooth muscle cells, and as having the potential to be stimulated by PDGF and neurotrophins capable of binding NGFR.

In our study of biopsies obtained from diseased human kidneys, areas of tubulointerstitial injury were accompanied by dramatic increases in the numbers of actin$^+$ cells. Such cells could be found in areas of active inflammatory injury, such as that occurring in allograft rejection and pyelonephritis, as well as in the areas of "nonspecific" chronic tubulointerstitial injury that are often encountered in biopsies of older individuals showing principally microvascular disease and nephrosclerosis (Figure 1B) and focally in biopsies of many individuals with glomerulonephritis. In cases of apparently active inflammatory injury, areas devoid of actin$^+$ cells also could be identified (Figure 1E). This diminution of actin expression could be due to the down-regulated expression of actin by interstitial cells in the area of injury or the temporary loss or displacement of such cells resulting from inflammatory cell infiltration. Individual actin$^+$ interstitial cells occasionally present in such areas of inflammation might represent residual constituent interstitial cells or could also represent interstitial

fibroblasts migrating into an area of injury that had lost such cells earlier in the course of the inflammatory injury (Figure 1D and F).

A particularly noteworthy finding was that, in glomerulonephridites with organizing crescents, a trail of actin$^+$ interstitial cells at times could be seen extending from the periglomerular interstitium into the urinary space, presumably migrating in response to specific chemotactic stimuli. Such cells were not encountered within cellular crescents in glomeruli with intact Bowman's capsules in the small number of glomeruli exhibiting these features that were included in this study. Studies in experimental animals have suggested that resolving crescents contain fibroblasts of interstitial origin (39,40). Studies of the composition of organizing glomerular crescents in humans have demonstrated the presence of collagen types known to be synthesized by interstitial cells but not normally by cells intrinsic to the glomerular tuft; this too has suggested that interstitial cells might migrate into severely damaged glomeruli, particularly at sites where Bowman's capsule has been disrupted (41–43). This study provides morphologic evidence for this scenario in human crescentic glomerulonephritis, although it must be recognized that a static biopsy study such as ours is insufficient to establish a chronologic course of injury.

One further consequence of the finding of extensive expression of muscle filaments in renal interstitium is that it suggests a hypothesis that the whole kidney may function as a contractile organ in response to certain stimuli or injury, with resultant consequences for RBF and pressure. Such a hypothesis is at present only weakly supported by the data presented, in view of the absence of organized networks of contractile cells such as occur in the walls of blood vessels or the muscularis mucosae of hollow organs, which enable these structures to contract in a functional manner. Further, it is not known if actin-expressing interstitial cells are capable of developing the complex intercellular junctions with each other that might facilitate such organized activity; future ultrastructural studies of this process may resolve this particular issue. Nonetheless, an intriguing study by Nagle *et al.*, using a model of ureteral obstruction in the rabbit in which an increase in smooth muscle-like interstitial cells occurs, has demonstrated that, under these conditions, the kidney may indeed demonstrate increased whole-organ contractility (44). Methods that might measure the contractility of human kidneys and relate them to the smooth muscle-like features of the interstitial cell populations would therefore be of great interest but have yet to be devised.

In conclusion, these studies characterize an important renal cortical interstitial cell population that participates in chronic tubulointerstitial injury as myofibroblast-like. We believe that the phenotypic characterization of interstitial cells *in vivo* should lead to a better understanding of the mechanisms of acute and chronic tubulointerstitial injury and should lead to better approaches to isolate and characterize such cells for *in vitro* studies of their response to physiologic and pathologic stimuli.

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