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F-actin fiber distribution in glomerular cells: Structural and functional implications

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F-actin fiber distribution in glomerular cells: Structural and functional implications.

Background. Glomerular distention is associated with cellular mechanical strain. In addition, glomerular distention/contraction is assumed to influence the filtration rate through changes in filtration surface area. A contractile cytoskeleton in podocytes and mesangial cells, formed by F-actin–containing stress fibers, maintains structural integrity and modulates glomerular expansion. In this study, the glomerular cell distribution of F-actin and vimentin filaments was studied in normal control and nine-month streptozotocin-diabetic rats. Results in situ were compared with observations in tissue culture.

Methods. Microdissected rat glomeruli were perfused to obtain a physiological 25% glomerular expansion over the basal value. Fixation was done without change in glomerular volume. Dual fluorescent labeling of F-actin and vimentin was carried out, and samples were examined by confocal laser scanning microscopy.

Results. The podocyte cell bodies and their cytoplasmic projections, including the foot processes, contained bundles of vimentin-containing fibers. Except for a thin layer at the base of foot processes, they did not demonstrate F-actin. While mesangial cells in culture had F-actin as long stress fibers resembling tense cables, mesangial cells stretched in situ contained a maze of short tortuous F-actin fibers organized in bundles that often encircled vascular spaces. This fibrillar organization was disrupted in diabetic glomeruli.

Conclusion. Mesangial cells, but not podocytes, contain a cytoskeleton capable of contraction that is disorganized in long-term diabetes. Together with previous observations, the distribution of this cytoskeleton suggests that mesangial cell contraction may be involved in the redistribution of glomerular capillary blood flow, but not substantially in the modulation of glomerular distention. Disorganization of stress fibers may be a cause of hyperfiltration in diabetes.

Direct measurements of compliance in isolated microperfused rat glomeruli have demonstrated the great mechanical strength and marked elasticity of these structures. Glomeruli are capable of rapid changes in volume following slight perturbations in internal pressure [1]. Thus, in the presence of impaired afferent arteriolar autoregulation, transient increases in perfusion pressure are associated with glomerular expansion. As the transcapillary hydrostatic pressure difference reaches levels existing in normal glomeruli (35 to 40 mm Hg) [2, 3], volume increases to between 17 and 25% of its basal value [1]. Through imaging deconvolution, it has been shown that as the glomerulus expands the overall change in volume results from equivalent increases in capillary volume and in the volume of other glomerular structures, including the mesangial areas [1].

There is abundant evidence indicating that the cellular mechanical strain imposed by the recurrent episodes of glomerular expansion associated with altered hemodynamics is, at least in part, responsible for the progressive accumulation of mesangial extracellular matrix and eventual glomerulosclerosis [4–7]. In addition, it has been proposed that dynamic modulation of glomerular volume may be an important factor in the regulation of glomerular filtration through alteration of the capillary surface filtration area [8, 9]. Hence, it is important to define the elements that, by contributing to glomerular structural stability and rigidity, may modulate distention [8]. Although studies in isolated microperfused glomeruli suggest that the contribution of the cellular contractile activity to overall glomerular rigidity is small [1], other evidence obtained in tissue culture and isolated nonperfused glomeruli points to the antithesis. Mesangial cells in tissue culture demonstrate increases in cytosolic Ca^{2+} and intense contraction when exposed to vasoconstricting substances [10–15]. Similarly, the presence of vasoactive agents may also alter the size of freshly isolated glomeruli in suspension [16–20], suggesting that cell contractility in situ, either static (isometric) or dynamic (isotonic), may limit glomerular expansion.

Stress fibers are fundamental to the contractile appara-

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tus of nonmuscle cells, such as fibroblasts. These fibers consist of a highly organized array of thick contractile bundles that are formed by filamentous actin (F-actin) and myosin II, which is typically prominent in cells that are exposed to high-tensional forces or shear stress [21]. At one end, stress fibers insert into the cytoplasmic side of the focal adhesion plaques where the external face of the cell is firmly attached to the extracellular matrix. At the other end, these fibers insert into a second focal adhesion or merge with the perinuclear actin cytoskeleton [22]. A prominent array of stress fibers has been demonstrated in both glomerular mesangial and epithelial cells in tissue culture [23, 24], supporting the postulate that the contractility of these cells importantly restricts volume expansion and maintains glomerular architectural organization.

Studies of isolated suspended glomeruli from diabetic animals have demonstrated impaired glomerular contractility in response to vasoconstrictive agents [25, 26]. In addition, reduced cell contractility has also been shown in cultured mesangial cells after exposure to high glucose concentrations [26–28]. Interestingly, prolonged culture of mesangial cells in high glucose concentrations induces disassembly of F-actin and loss of stress fibers [24, 29]. Therefore, it has been postulated that mesangial cell hypocontractility is a major alteration responsible for the characteristic glomerular hyperfiltration of diabetes [24].

It is apparent that the cytoskeletal organization of glomerular cells, normally under a narrowly defined degree of stretch, cannot be adequately evaluated in the absence of intraglomerular pressure, that is, in nonperfused, collapsed glomeruli. In this study, the distribution of the F-actin cytoskeleton has been investigated ex vivo in freshly isolated, microperfused glomeruli, subjected to the range of intracapillary pressure and volume expansion that are expected to occur in situ under physiological conditions. In addition, these results have been compared with the organization of actin in stress fibers in mesangial cells in tissue culture. As reference, the expression and distribution of vimentin have also been studied in glomerular cells in situ and mesangial cells in tissue culture. Vimentin is a widely distributed intermediate filament protein expressed by many cells of mesodermal origin, sometimes only transiently during development. Vimentin networks are highly elastic, apparently providing mechanical stability to the cell [30]. The findings in this study delineate the possible roles of glomerular mesangial and visceral epithelial cells in the control of glomerular expansion under normal and diabetic conditions and also the relevance of cytoskeletal studies in tissue culture.

METHODS

Materials

The glomerular perfusion solution was composed of a specially formulated RPMI-1640 (GIBCO BRL, Grand Island, NY, USA). This modified solution lacked glucose and glutamine and was buffered with 25 mmol/L HEPES. Before perfusions, the following were added: 5 mmol/L D-glucose, 2 mmol/L L-glutamine, 10 mg/mL bovine serum albumin (BSA), 100 U/mL penicillin, 100 μg/mL streptomycin, 20 μmol/L sodium orotate, and 1% ITS+ Premix® (Collaborative Biomedical Products, Oak Park, MA, USA) providing per milliliter, 625 μg selenious acid, 5.35 μg linoleic acid, and 6.25 μg insulin. In addition, 2 mmol/L glycine, 0.5 mmol/L reduced glutathione, 1 mmol/L sodium pyruvate, and 50 μmol/L adenine were also added to protect the tissue from anoxic injury. As growth medium for the tissue culture experiments, 8 mmol/L D-glucose, 0.14 mmol/L glutamine, and 20% NuSerum (Collaborative Research, Bedford, MA, USA) were added to the specially formulated RPMI.

The cross-linking agent dithio-bis(succinimidylpropionate) (DSP) and Triton® X-100 (Surfac-Amps®) were obtained from Pierce (Rockford, IL, USA). Mouse monoclonal antivimentin antibody (Dako, Carpinteria, CA, USA) as primary antibody and polyclonal Cy3-conjugated goat anti-mouse IgG (Amersham, Arlington Heights, IL, USA) as secondary antibody were used for visualization of vimentin filaments. The specific fluorescence labeling of F-actin was carried out with Oregon Green 488-phalloidin (Molecular Probes, Eugene, OR, USA). Samples for confocal microscopy observation were mounted in ProLong™ Antifade (Molecular Probes).

Animals and isolation of glomeruli

Male Munich-Wistar rats (WM/Sim strain; Simonsen Laboratories Inc., Gilroy, CA, USA) weighing 250 to 300 g were used. We chose this strain because their glomerular intracapillary pressure has been well documented. In the diabetic group, insulin deficiency was induced by the intravenous injection of 55 mg/kg body weight of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA) dissolved in acidified 0.9% NaCl. Induction of diabetes was documented by the measurement of blood glucose 48 hours after streptozotocin injection. At the time of the experiment, blood glucose levels varied between 334 and 436 mg/100 mL. Animals did not receive insulin replacement. All diabetic animals were studied nine months after induction of the disease. Age-matched normal animals served as controls.

In all experiments, rats were anesthetized with an ether/O_2 mixture. Perfusion of the kidneys in situ was carried out for two minutes at a pressure similar to the animal's mean arterial pressure using a perfusate at 4°C. The solution used for perfusions and for glomerular studies was filtered through a 0.22 μm pore size filter, equilibrated for 30 minutes in a hollow-fiber oxygenator (CellPharm®; CD Medical, Inc. Miami Lakes, FL, USA) with 5% CO_2/95% O_2 at 37°C, pH 7.40. At the end of the kidney perfusion period, 4 mL of 60 to 70 mg/mL dye-

complexed BSA (Coomassie Blue and Evans Blue) dissolved in the same perfusate solution was infused to identify well perfused, nonsclerotic glomeruli and to facilitate arteriolar visualization during tissue dissection. To minimize glomerular size heterogeneity, only outer cortical glomeruli were studied [31]. Glomeruli with intact afferent arterioles were dissected, and their Bowman's capsules were removed at 4°C. To insure that glomeruli of uniform viability were studied, experiments were aborted if the period between removal of the kidney and initiation of glomerular perfusion and incubation at 37°C exceeded 50 minutes. Thus, only one glomerulus was studied from each rat. In experiments in which diabetic and age-matched animals were used, a tissue specimen was also obtained from the same kidney for light microscopic evaluation of glomerular damage. After fixation in 3.7% paraformaldehyde in phosphate-buffered saline (PBS), specimens were processed for periodic acid-Schiff staining of 4 to 5 μm sections.

Glomerular perfusion

Glomerular perfusion was carried out using methods similar to those previously described [1]. After transfer of the dissected glomerulus to a temperature-regulated observation/incubation chamber mounted on an inverted microscope (Diaphot; Nikon Inc., Garden City, NY, USA) the afferent arteriole was drawn into a holding pipette and cannulated with a matching perfusion pipette at a temperature of 4°C. Next, a 2 μm outer diameter pipette was placed at a point close to the bifurcation of the afferent arteriole in the mesangial region for the direct measurement of hydrostatic pressure as proximal intraglomerular pressure (PIP) by the Landis technique [32] using a pneumatic transducer tester (Model DPM-IB; Bio-Tek Instruments Inc., Winooski, VT, USA). The micropipette assembly also included a fourth pipette, placed between the perfusion and pressure pipettes, that virtually eliminated the large void volume from the perfusate container to the tip of the perfusion pipette. This arrangement facilitated the rapid exchange of perfusion solution for fixative buffer, without disrupting perfusate flow or modifying intraglomerular pressure. Glomeruli were examined with a high-resolution 3-CCD video camera (Sony DXC-750; Sony Corp. of America, Park Ridge, NJ, USA) with continuous quantitative evaluation of volume by image analysis (Image-1; Universal Imaging Co., West Chester, PA, USA). This was carried out on the optical plane that provided the largest glomerular surface area using a computer-assisted best fit of an ellipse that encompassed the glomerular perimeter and rotated on its longest axis (Metamorph 3.6; Universal Imaging Co.).

Upon raising the chamber's temperature to 37°C, glomerular perfusion at a low flow with PIP of less than 10 mm Hg was carried out during an eight-minute stabilization period. Following equilibration, perfusion was interrupted, and the basal glomerular volume (volume at zero internal pressure) was measured. Perfusion was then resumed at a flow that provided an intraglomerular pressure that resulted in 25% volume expansion. In all of the glomeruli studied, the PIP required for this degree of expansion was between 52 and 58 mm Hg, which approximates a mean transcapillary hydrostatic pressure difference of 34 to 38 mm Hg [1]. After documenting a stable glomerular volume, the perfusate was switched to a solution of 3.7% paraformaldehyde in PBS, pH 7.40, without altering the flow rate or the degree of glomerular expansion. Complete fixation was achieved after subsequent 5- and 10-minute perfusions at 37°C and 4°C, respectively.

Tissue culture

Mesangial cells were obtained from our previously characterized cloned cell line, $16KC_2$, derived from outgrowths of Fischer rat glomeruli [33]. In addition, primary cultures of rat mesangial cells, obtained from sieved glomeruli [33], were examined at the time of their first passage. Cells were seeded (3×10^4 cells/chamber) into glass chamber slides (Lab-Tek II; Nalge-Nunc, Naperville, IL, USA) and used for cytoskeletal studies three-days postseeding when cultures were 30% confluent.

Immunocytochemistry

Methods for cross-linking and permeabilization of glomeruli were carried out by methods similar to those previously described for cultured cells [34]. Paraformaldehyde-fixed glomeruli were cross-linked with 1 mmol/L DSP, dissolved in Hank's balanced salt solution buffer, pH 7.4, and treated with 100 mol/L 1,4-piperazinediethanesulfonic acid buffer, pH 6.9, containing 1 mol/L egtazic acid (EGTA), 4% polyethylene glycol 800, 1% Triton, and 1 mol/L DSP. Following embedding in 5% agarose, two 50 μm Vibratome sections from each glomerulus were blocked with a 1:100 dilution of goat serum in PBS. Antibody reactions were carried out overnight at 4°C with primary antibody (1:1000 dilution) and for two hours at room temperature for the Cy3-conjugated secondary antibody (1:100 dilution) dissolved in PBS containing 1% BSA. Following washes in PBS, sections were incubated for 20 minutes at room temperature with 2.5 U/mL Oregon Green phalloidin, thus resulting in dual fluorescence labeling of vimentin and F-actin. Specimens of cultured mesangial cells were processed as described for glomeruli, except that cross-linking was not carried out and 0.2% Tween was used for permeabilization. The specificity of the immunolabeling was established with nonimmune IgG.

In confocal microscopy, fluorochromes were excited by a krypton argon laser with a pinhole size of 90 using excitation wavelengths of 488/588 nm, a 510/580 double

dichroic mirror and a 515 to 545 band pass fluorescein filter together with a 590 nm long pass filter. An MRC 1024 scan head (Bio-Rad Labs, Hercules, CA, USA), attached to an inverted microscope (Axiovert 100; Carl Zeiss, Inc., Thornwood, NY, USA), was used to obtain sequential dual-color images with an optical section height of 0.5 μm and the appropriate filter blocks to ensure that there was no bleed through between channels. Objectives of either NA 1.4 × 63 or NA 1.4 × 100 were used. Images were subsequently enhanced by edge sharpening using Adobe Photoshop version 4.0 software (San Jose, CA, USA) and printed on a Codonics NP1600 (Middleburg Heights, OH, USA) dye sublimation printer.

RESULTS

Cytoskeleton in expanded, normal glomeruli

Basal glomerular volume, after incubation at 37°C for eight minutes, was $1.013 \pm 0.270 \times 10^6$ μm^3 (mean, SD, $N = 5$). Without exceeding physiological limits of intraglomerular pressure, a 25% expansion was achieved in all of the glomeruli studied without evidence of mechanical disruption of the tuft. In all instances, this degree of expansion was maintained for less than three minutes before perfusion with fixative was initiated. Fluorescent labeling with increasing concentrations of antivimentin antibody or Oregon Green 488 phalloidin only resulted in higher background staining, without altering the cellular distribution of the labels. Results were virtually identical in all of the glomeruli successfully perfused and examined.

Thin optical sections of the glomerular tuft demonstrated intense fluorescence labeling of vimentin in podocyte cell bodies and their processes (Fig. 1A). This arrangement provided a network of vimentin-containing bundles enveloping the entire capillary circumference, except at the perimesangial area where vimentin was absent. At the peripheral capillary wall, vimentin was distributed interspersed with F-actin as a continuous layer (Fig. 1B). Z-axis projections displayed vimentin as a complex mesh of delicate fibers, most evident in a perinuclear distribution within the cell body and in the primary processes (Fig. 2A). These fibers packed together into a continuous dense bundle along the secondary and subsequent subdivisions of the cellular extensions, finally reaching the foot processes (Figs. 1A and 2A). Interestingly, except for the base of foot processes (discussed later in this article), there was no detectable F-actin in the podocyte cell bodies or in their extensions, including the finest branches enwrapping the capillaries.

Thin optical sections of the mesangial areas demonstrated dense F-actin labeling, mostly forming coarse, sinuous strips, and clumps that were separated by serpentine, nonfluorescent "channels" that likely represented the "photo-negative" image of extracellular areas of mesangial matrix (Fig. 1). In addition, some of the areas of F-actin were composed of bundles of short fibers that demonstrated an organized distribution. The orientation of these fibers was most clearly evident in tridimensional reconstructions of the mesangial regions (Fig. 2B). Here, the actin fibers were characteristically arranged as a maze of tortuous, coarse elements, irregular in shape and often encircling vascular spaces. Notably, scant vimentin labeling was demonstrated in mesangial regions, and when present, most staining could be traced to podocyte extensions and foot processes (Figs. 1A and 2B).

The peripheral capillary wall demonstrated two distinct, well-organized cytoskeletal components. One was the dense vimentin-containing bundles in podocyte extensions and foot processes described previously (Figs. 1 and 3A). The second was formed by a thin layer of tortuously branching, slender filaments of F-actin, interspersed with the vimentin at the base of the foot processes (Fig. 1), which provided an additional cytoskeletal envelope throughout the capillary wall (Fig. 3).

Cytoskeleton in mesangial cells in tissue culture

In contrast to mesangial cells in situ, both cloned and primary mesangial cells in subconfluent cultures demonstrated a well-developed lace of thin vimentin-containing fibers that extended over the entire cell body without particular orientation (Fig. 4).

Both types of mesangial cells studied in culture also possessed abundant F-actin–containing fibers demonstrating the characteristic disposition of stress fibers (Fig. 4). These were arranged as straight, cable-like cords transversing the cytoplasm in multiple directions, but preferentially oriented along the main cellular longitudinal axis (Fig. 4A). Several of these cords frequently converged at one single point at the edge of the cytoplasmic extensions, presumably representing focal adhesion plaques.

Cytoskeleton in expanded diabetic glomeruli

After its intrarenal injection during perfusion of the kidney in situ, the amount of albumin-complexed dye present in glomeruli was used as an indicator of the level of glomerular perfusion in vivo. To avoid glomeruli with significant sclerosis, only those demonstrating abundant quantities of dye within their capillaries were microdissected for study.

Diabetic glomeruli demonstrated a basal volume, after incubation at 37°C for eight minutes, that was not significantly greater than age-matched controls (diabetic, $3.298 \pm 0.270 \times 10^6$ μm^3, $N = 5$; control, $2.972 \pm 0.866 \times 10^6$ μm^3, $N = 4$). In diabetic glomeruli, the location of vimentin and F-actin among the different cellular components was similar to that observed under normal conditions. Podocytes demonstrated vimentin arranged in fibers, as described previously in this article, although the bundles of vimentin present in the cell's extensions

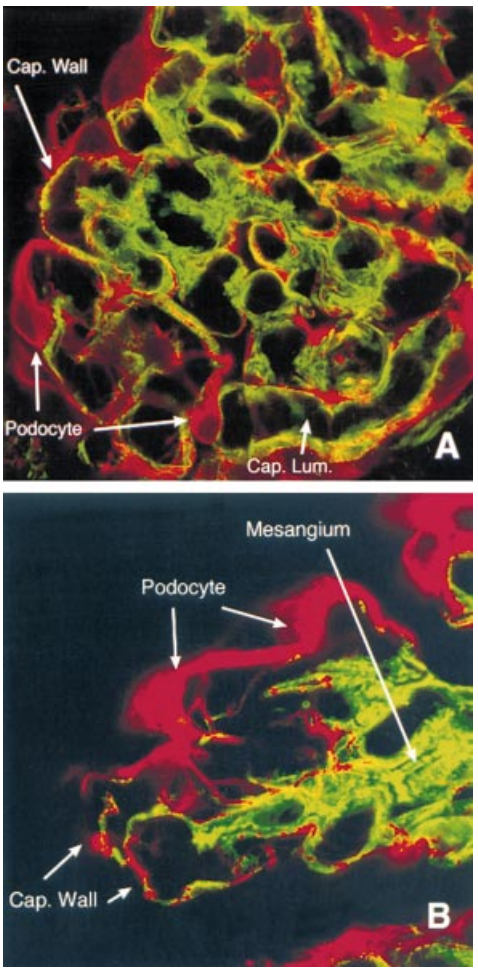

Fig. 1. Cellular distribution of vimentin- and F-actin–containing fibers in a distended glomerulus. A glomerulus was fixed during perfusion at a pressure to induce a 25% expansion over its basal, unperfused volume. The presence of F-actin (green) and vimentin (red) is shown in podocytes and mesangial areas by confocal microscopy. (*A*) One-half micrometer optical section demonstrating the podocyte's cytoskeletal components and their distribution in relationship to the capillary lumina (Cap. Lum.) and capillary wall (Cap. Wall). Strands of nonlabeled matrix are mesangial "channels." Magnification ×63. (*B*) Detail of the interspersed distribution of F-actin and vimentin along the peripheral capillary wall. Magnification ×160.

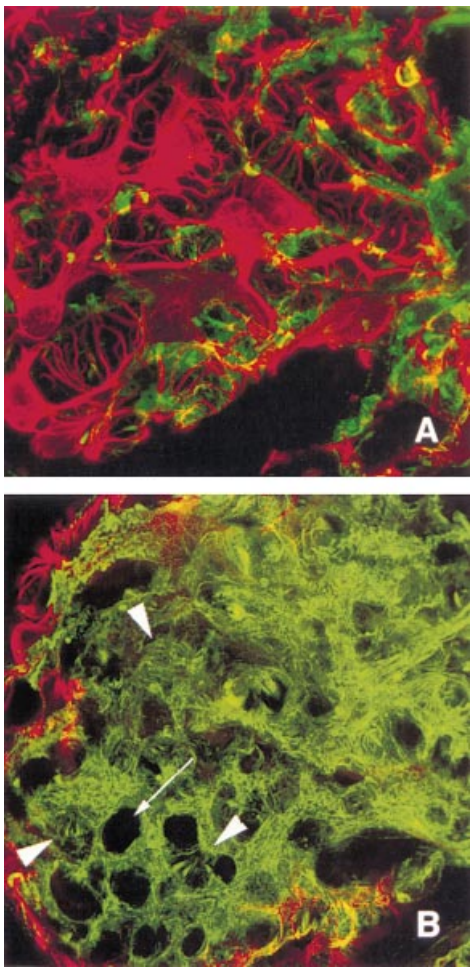

Fig. 2. Podocyte and mesangial distribution of vimentin- and F-actin–containing fibers in a distended glomerulus. A glomerulus was perfused and fixed as in Figure 1. The location of F-actin (green) and vimentin (red) is shown. (*A*) Projected image of a 10 μm thick Z-series composed of 20 0.5 μm optical sections demonstrating the distribution of vimentin in the cell body and processes of podocytes enveloping peripheral capillaries. (*B*) Projected image of a 6 μm thick Z-series composed of 12 0.5 μm optical sections demonstrating the mesangial distribution of F-actin–containing fibers and their relationship to a mesangial capillary (arrow). Instances in which F-actin fibers envelop mesangial capillary lumina are shown (arrowheads). Magnification ×100.

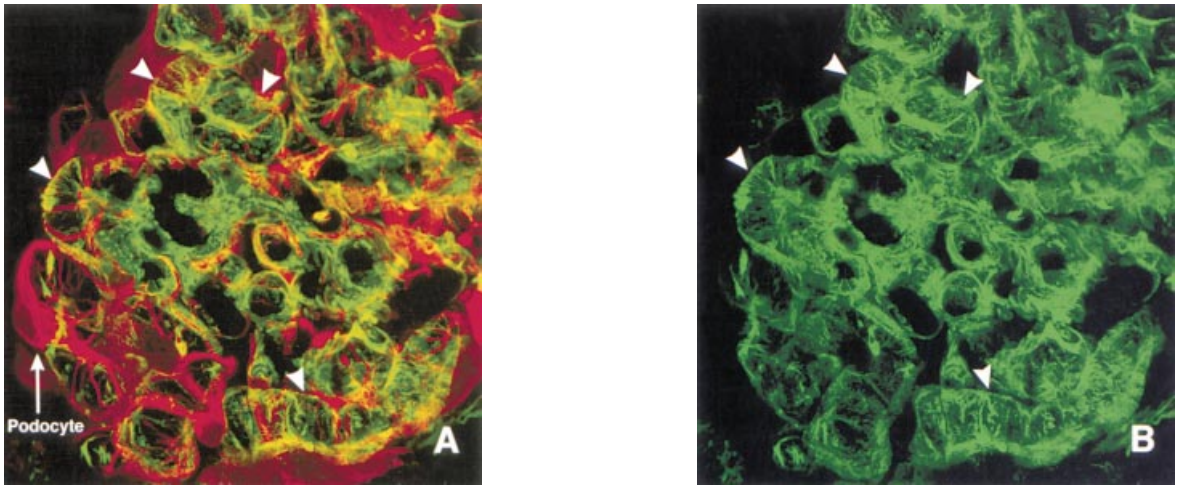

Fig. 3. Distribution of F-actin– and vimentin-containing fibers on the capillary wall of a distended glomerulus. A glomerulus was perfused and fixed as in Figure 1. (*A*) Projected image of a 10 μm thick Z-series composed of 20 0.5 μm optical sections demonstrating the distribution of vimentin (red) in podocytes' processes enveloping peripheral capillaries and their relationship to F-actin (green) fibers on the capillary wall (arrow heads). (*B*) Single-channel collection of the same image in which vimentin labeling has been excluded to better demonstrate the F-actin cytoskeleton of the peripheral capillary wall. Magnification ×63.

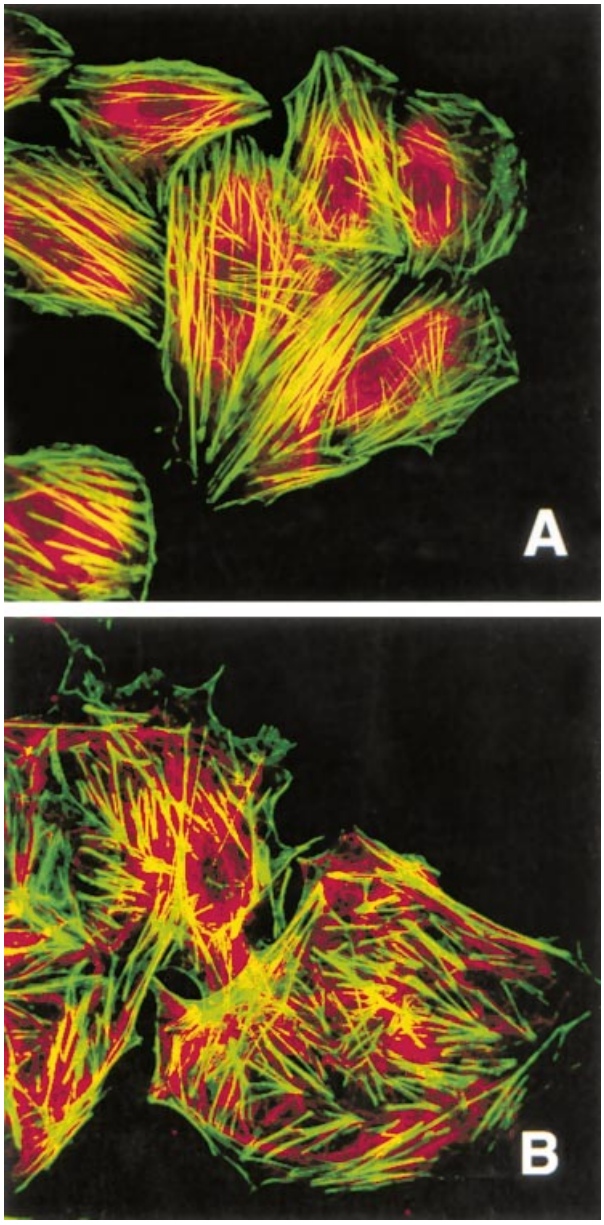

Fig. 4. Distribution of F-actin– and vimentin-containing fibers in mesangial cells in tissue culture. Subconfluent cultures of a clone line of mesangial cells or a first-passage culture of rat primary mesangial cells were specifically labeled to demonstrate their cytoskeletal characteristics. (*A*) Group of cloned cells demonstrating F-actin–containing stress fibers (green) and the network of vimentin-containing fibers (red) distributed surrounding the cell nucleus (unlabeled). Magnification ×100. (*B*) Primary culture cells, identically processed, demonstrating the same cytoskeletal components.

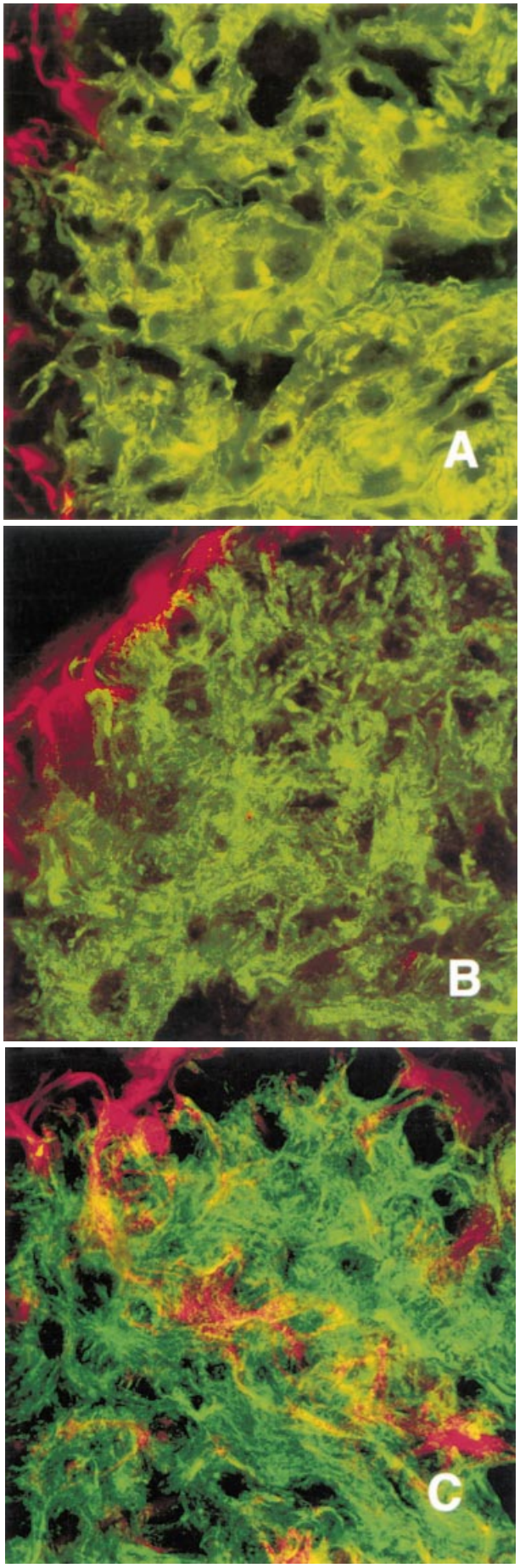

Fig. 5. Mesangial cell distribution of F-actin–containing fibers in distended glomeruli from diabetic animals. Glomeruli from nine-month diabetic animals (*A* and *B*) and age-matched controls (*C*) were perfused and fixed as in Figure 1. The location of F-actin (green) and of vimentin (red) in the podocytes extensions is demonstrated. Images are projections of a 4 μm thick Z-series composed of 8 0.5 μm optical sections demonstrating the paucity of discrete F-actin–containing fibers (A) and their disorganization in areas in which they were present (B), as compared with controls (C). Magnification ×125.

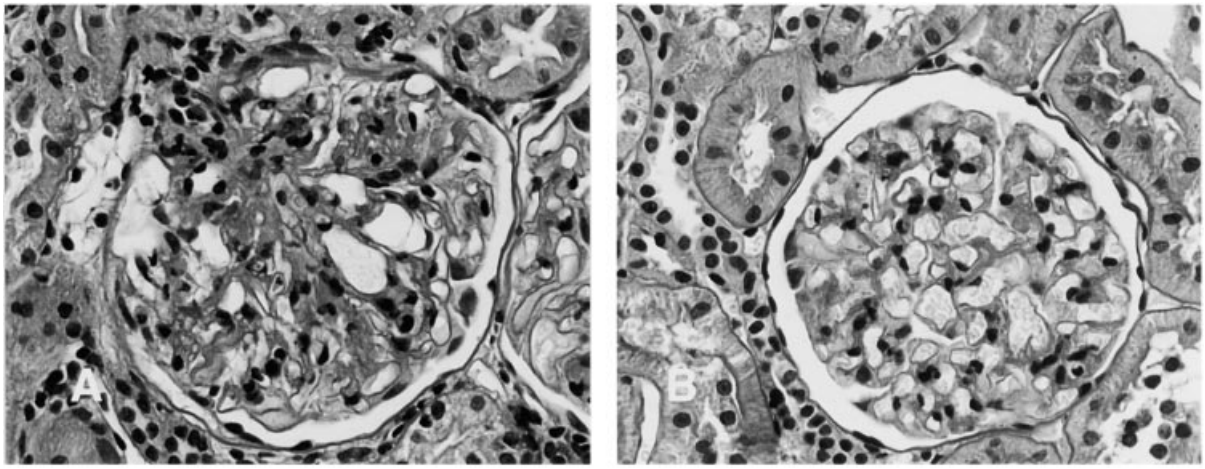

Fig. 6. Glomerular disease in diabetic animals. Light microscopic analysis was carried out in periodic acid-Schiff–stained sections obtained from the same kidneys that were used for glomerular microdissection. Shown is a representative glomerulus from a diabetic animal demonstrating moderate mesangial expansion (*A*) as compared with disease-free glomeruli in age-matched controls (*B*). Original magnification ×40.

were less dense, thinner, and far more irregular than in normal glomeruli (data not shown). F-actin was, again, concentrated in the mesangial regions but with marked alterations in its disposition. As compared with age-matched controls, F-actin–containing fibers in diabetic glomeruli were virtually absent or ostensibly disorganized (Fig. 5). Tridimensional reconstructions of the mesangial regions still containing fibers demonstrated only lumps of F-actin and short, coarse fibers lacking any specific organization (Fig. 5B).

Light microscopic evaluation of glomerular damage in the same diabetic kidneys used for glomerular perfusion demonstrated no disease or mild to moderate mesangial expansion in 5 to 10% and 60 to 70% of the glomeruli examined, respectively (Fig. 6). The rest of the glomeruli presented various degrees of focal and segmental sclerosis.

DISCUSSION

In this study, to reveal the orientation and distribution of the principal fibrillar elements that may provide the cellular component of the glomerular structural strength, glomeruli were fixed while expanded under physiological pressures. Previous electron microscopy studies of the glomerular cytoskeleton had been carried out in incubated glomeruli in suspension [35], or in specimens of perfusion-fixed kidneys, either while in situ or after isolation of the kidney [36–39]. Although renal perfusion fixation results in glomerular distention, the intraglomerular pressure and the degree of glomerular expansion obtained are uncertain because of the variability of afferent arteriolar autoregulation. Thus, to our knowledge, this represents the first study combining isolated glomeruli, fixed under physiological conditions of expansion, with dual-label immunocytochemistry utilizing tridimensional reconstruction of confocal images.

As in previous studies, we again demonstrated that podocytes possess abundant vimentin-containing intermediate filaments that coalesce as dense bundles into the cells' processes [36, 40–42]. Following the primary and secondary processes, vimentin bundles from the same cell frequently spread out over two or more neighboring capillary loops. In contrast to previous findings [36], these vimentin filaments further arborize into the small branches, finally extending into the foot processes, reaching their base. At this location, they are interposed with delicately ramifying F-actin filaments that envelop the capillary wall. In previous immunogold electron microscopic studies utilizing antiactin antibodies that react with both globular (unpolymerized) and F-actin, actin was demonstrated along the entire length of the foot processes in high density [36]. In the present study, utilizing a specific label for F-actin, the capillary wall cover provided by these filaments appears to be limited to a thin layer at the base of the foot processes. Notably, except for this juxtacapillary layer, F-actin was absent in the rest of the podocyte, including all of its major processes. Interestingly, the contractile properties and glomerular volume-reducing ability attributed to the podocyte [39, 43] have been principally based on the presence of abundant stress fibers containing F-actin in primary cultures of glomerular epithelial cells [44]. Our results suggest that visceral epithelial cells in tissue culture express an actin cytoskeleton that is not present in situ. Podocytes in situ enjoy a rich elastic network of vimentin fibers that are probably important in providing structural stability. However, although strategically located to limit distention and separation of adjoining capillary loops, podocytes appear to lack a significant contractile system of stress

fibers capable of actively modulating glomerular expansion. Furthermore, it is questionable whether the delicate network of F-actin enveloping the capillaries is capable of limiting their distention significantly. The location of this F-actin is consonant with its hypothesized role in active alteration of the glomerular ultrafiltration coefficient by contractile deformation of the foot processes and the interspersed filtration slits [35]. However, intrarenal infusion of angiotensin II in vivo does not alter the width of foot process, the total filtration slit length, or the mean width of individual filtration slits [45]. Although the image resolution of the techniques used in this study did not permit fine delineation of the endothelial cell cytoskeleton, it is unlikely that it may be important as a capillary contractile apparatus because previous electron microscopic evaluations have demonstrated only small amounts of actin [36].

This and other previous studies have documented that mesangial cells in culture possess a dense array of stress fibers that transverse the cell body as long tense cables joining distant focal adhesions, thereby implying the presence of high tensional forces [24, 29]. In addition, since these cells vigorously contract when exposed to vasoconstrictive agents [10, 11], some investigators have concluded that their isometric and isotonic contraction are important to the maintenance of glomerular structural integrity and fine regulation of filtration surface area by reduction of glomerular volume [8, 9]. In contrast, more recent studies in situ have shown that the infusion of angiotensin II in the rat does not alter glomerular volume or peripheral capillary surface area, although decrement of the ultrafiltration coefficient is consistently produced [45–47]. This work demonstrates an abundance of F-actin fibers in the mesangial areas, which represent by far the bulk of the glomerular F-actin. Therefore, if a significant contractile apparatus in the glomerulus exists, it most likely resides in the mesangial cell. However, the appearance of these fibers is quite different from that shown in mesangial cells in tissue culture. Even though mesangial cells were stretched in situ to a physiological level during glomerular expansion, their F-actin fibers appeared as curvilinear or undulating bundles of diverse diameter, some of them reminiscent of the microfilaments interconnecting opposing mesangial angles in the juxtacapillary mesangial region previously described by Kriz et al [48]. More often, in image reconstructions of the mesangium, these fibers were seen as sinusoidal bundles completely encircling vascular spaces. This location probably corresponds to the recently described "mesangial loops," comprising regions of the mesangial tree where the mesangium completely surrounds a capillary [49]. These mesangial loops contain cytoplasmic processes of mesangial cells with prominent bundles of microfilaments that envelop nearly 15% of all capillary branches within a given glomerulus. It has been theorized that dynamic contraction of these processes may unevenly redistribute intraglomerular blood flow [49]. The corollary of this would be that the contractile state of the mesangial cell may alter plasma flow and ultrafiltration rate in a specific group of downstream peripheral capillaries, with little effect on total glomerular volume.

Functional studies have demonstrated that the integrity of the mesangial cell is essential for the characteristic decrease of the ultrafiltration coefficient caused by the contractile action of angiotensin II [47]. Our previous studies in isolated perfused glomeruli demonstrated that cellular tone was responsible for only 4.1% of the total restriction to volume expansion that resulted from increased intracapillary pressure [1]. This evidence, coupled with our present findings, suggests that while the contractile apparatus of the mesangial cell may be involved in the regulation of glomerular filtration, it only plays a minor role in the modulation of glomerular distention. Consequently, increased mesangial cell tone is unlikely to ameliorate the mechanical strain induced by increased intraglomerular pressure [4, 6].

The observations made in this study of the F-actin and vimentin distributions in mesangial cells in tissue culture and in situ clearly illustrate the differential expression of cytoskeletal components in these circumstances. Therefore, regarding the cytoskeletally related functional attributes of glomerular cells, direct extrapolations of observations in tissue culture to the situation in situ must be interpreted with caution. This is particularly evident when assessing the contractile ability of podocytes where these cells express a rich and well-organized system of stress fibers in tissue culture [24, 44], but not in situ. In regard to the increased expression of vimentin-containing intermediate filaments in mesangial cells in tissue culture, this represents an early change, probably occurring during the initial glomerular cell outgrowth since this is already evident in first passage cultures.

Finally, this study demonstrates that in long-term diabetes, there is a significant loss and disorganization of the F-actin–containing stress fibers in mesangial cells. Although well-perfused glomeruli in vivo were selected for study, these diabetic kidneys demonstrated mild to moderate disease in most glomeruli. Therefore, it is not known at this point whether this cytoskeletal alteration is specific to diabetes or whether it is an expression of incipient glomerulosclerosis. Notably, as previously specified, mesangial cells exposed to high glucose concentrations in tissue culture also demonstrate a similar F-actin disassembly with loss of stress fibers [24, 29]. Therefore, the glucose-induced cytoskeletal alterations of mesangial cells in culture appear to recapitulate changes occurring in situ, suggesting an impairment of contractility in these cells of which consequence is the defective control of glomerular perfusion and hyperfiltration in the intact animal.

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