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CLINICAL INVESTIGATION

Developmental patterns of PDGF B-chain, PDGF-receptor, and α-actin expression in human glomerulogenesis

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Developmental patterns of PDGF B-chain, PGDF-receptor, and α-actin expression in human glomerulogenesis. Expression of PDGF B-chain and the PDGF receptor β-subunit (PDGFRβ) is detected immunocytochemically during the development of glomeruli in human kidneys of 54 to 105 days gestational age. During the early stages (vesicular, comma-shape and S-shape) of glomerulogenesis, PDGF B-chain is localized to differentiating epithelium of the glomerular vesicle, while PDGFRβ is expressed in the undifferentiated metanephric blastema, vascular structures, and interstitial cells. During this stage PDGF may be acting as a paracrine growth factor and as a chemoattractant acting to recruit mesangial progenitor cells into the developing glomerulus. As the glomerular tuft forms, both PDGF B-chain and PDGFRβ can be detected in an arboreal pattern radiating from the hilus of the glomerular tuft. Immunocytochemical studies using markers specific to endothelium (Ulex europaeus I lectin, Factor VIII related antigen), and smooth muscle (α-smooth muscle actin), indicate that the PDGF B-chain and PDGFRβ are both expressed primarily by mesangial cells. During this stage, PDGF may be acting primarily to provide an autocrine factor to mediate further mesangial cell proliferation. Glomerular expression of α-smooth muscle actin is limited to later stages of glomerulogenesis; at these stages the pattern of expression is similar to that of PDGF-B chain and PDGFRβ. The upregulation of mesangial PDGF, PDGFRβ, and α-smooth muscle actin expression that has been identified in some disease states in both humans and experimental animals appears to represent a recapitulation of this normal developmental process.

The development and maturation of the mammalian glomerulus progresses through orderly stages beginning with differentiation from metanephric blastema and proceeding through "vesicle," "comma-shape," "S-shape" and fully differentiated glomerular stages [reviewed in 1–3]. Relatively little is known about the molecules that influence the multiple events involved in this process of cell proliferation, differentiation and maturation. Recent studies have identified potential roles for specific matrix proteins (such as laminin) [4], matrix-binding integrins [5, 6], and cell adhesion molecules (N-CAM [7, 8], uvomorulin [9]) in modulating specific segments of the developmental sequence. The role for peptide growth factors has been addressed in studies which implicate insulin like growth factor I and II (IGF) [10], epidermal growth factor (EGF) [11], and transforming growth factor α (TGFα) [12] in this process, but, as recently reviewed by Brenner, a complete understanding of the importance of these molecules is not yet at hand [13].

Platelet-derived growth factor (PDGF) is a covalent dimer of two subunit chains designated A-chain and B-chain, which exists in three naturally occurring isoforms (that is, PDGF-AA, PDGF-AB and PDGF-BB). PDGF binds to cells via cell surface receptors which function as noncovalent dimers of two subunits, designated as PDGF receptor α-subunit (PDGFRα) and the PDGF receptor β-subunit (PDGFRβ), in such a way that cells expressing only PDGFRβ are able to bind only PDGF-BB and cells expressing PDGFRα are able to bind all forms of PDGF [14]. PDGF has been shown to be mitogenic for a variety of cell types, including smooth muscle cells, glomerular mesangial cells, and fibroblasts [15, 16]. PDGF may also function as a chemotactic factor for these cells as well as for neutrophils and monocytes. PDGF was originally isolated from platelets and was proposed to play a role in promoting connective tissue cell proliferation at sites of tissue damage and consequent platelet degranulation [15]. Although delivery of PDGF via platelets is still considered to be an important source of PDGF, many other cell types are also induced to synthesize PDGF A-chain and B-chain under pathological conditions associated with cell proliferation (such as, traumatic injury, inflammation, tumorigenesis) and it has been proposed that this locally produced PDGF plays a role in initiating or maintaining this proliferation [15].

One type of tissue injury where PDGF may play a major role is in glomerulonephritis. Several cell types present in an injured glomerulus may release PDGF, including renal endothelial cells, mesangial cells, infiltrating macrophages and platelets. In both experimental and human proliferative glomerulonephridites, PDGF and PDGF receptors have been shown to be upregulated [17–20]. A particularly dramatic example is the mesangial proliferative nephritis induced in rats with antibody to the Thy-1 antigen [17, 18]. The levels of PDGF B-chain and PDGFRβ increase more than eightfold at a time when mesangial cells are proliferating rapidly [17, 18]. Infusion of a blocking antibody against PDGF inhibits about 60% of this proliferation at day 4 after disease induction [21], a time when mesangial cell production of PDGF appears to be the principal source of this factor. This is consistent with the hypothesis that the proliferation is driven in part by autocrine production of PDGF by the mesangial cells in conjunction with an enhanced ability to bind PDGF-BB.

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Although much less is known about possible roles of PDGF in embryonic development, there is some indication that the PDGF/PDGF receptor system may also be involved in developmental processes. PDGFRα is expressed by the mesoderm from the time that it can first be identified as a distinct germ layer, and continues to be expressed by a subset of mesodermal derivatives [22]. Mouse embryos which are homozygous for a deletion of the PDGF receptor α-subunit [23] display multiple mesodermal abnormalities and die before birth [22]. By contrast, PDGFRβ (which is the form of the receptor which seems to be induced in a number of adult disease states) cannot be detected in extracts of whole mouse embryos until later in development [24], nor is the identity of the embryonic cells expressing this receptor known. In the present study, we report that PDGF B-chain and PDGFRβ are expressed by the immature glomerulus at specific stages of development. To identify the cells expressing PDGF receptor β-subunit we have employed immunocytochemical markers of endothelium [Factor VIII-related antigen (F_{VIII}), Ulex europaeus I lectin] and the smooth muscle-like glomerular mesangial cells (α-smooth muscle actin). We report that the pattern of expression of α-smooth muscle actin and of PDGF B-chain and PDGFRβ in differentiated fetal glomeruli closely resembles the pattern seen in response to mesangial injury and speculate on the roles that PDGF may be playing in these processes.

Methods

Source of tissue

Human fetal kidneys (N = 44) were obtained fresh from tissue examined after therapeutic abortions. Fifteen of these (estimated gestational age ranging from 54 to 105 days) were fixed in methyl Carnoy's (methacarn) solution (60% methanol, 30% chloroform, 10% acetic acid) and processed and embedded in paraffin according to conventional techniques. Twenty-five kidneys (estimated gestational age ranging from 54 to 105 days) were fixed overnight in cold 2% or 4% paraformaldehyde in phosphate buffer, transferred to 30% sucrose in 0.01 M phosphate buffer, equilibrated overnight at 4°C, and snap frozen in OCT compound (Miles, Inc., Elkhart, Indiana, USA). Four kidneys (estimated gestational age ranging from 57 to 84 days) were fixed overnight in cold periodate-lysine-paraformaldehyde (PLP) solution, washed, dehydrated through 90% ethanol, embedded in LR White resin (Ted Pella, Inc., Redding, California, USA), and polymerized overnight at 55°C.

Immunohistochemistry

Sections of methyl Carnoy's fixed tissue were deparaffinized with xylene and graded ethanols, blocked with 3% hydrogen peroxide, and washed with 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 subsequently processed using a streptavidin-biotin immunoperoxidase method with 3,3′-diaminobenzidine (with nickel chloride enhancement) as the chromogen. Sections were counterstained with methyl green.

Frozen sections of 2% or 4% paraformaldehyde fixed tissue were hydrated in PBS, blocked with 3% hydrogen peroxide and washed in PBS. The sections were incubated overnight at 4°C with antibody 7212, washed, and subsequently processed as above using a streptavidin-biotin immunoperoxidase method, counterstained with methyl green, dehydrated and coverslipped.

For all samples, a negative control consisted of substitution of the primary antibody with both irrelevant murine monoclonal antibodies, non-immune rabbit sera in the case of Factor VIII related antigen, and PBS. Isotype-matched irrelevant murine antibodies were specifically utilized as controls for PDGF B-chain, PDGFRβ, and smooth muscle α-actin. Positive internal controls in each biopsy consisted of actin-positive smooth muscle cells comprising the renal vasculature for the α-SM-1 antibody and arterial endothelial cells for Ulex lectin and anti-Factor VIII related antigen antibodies.

Antibodies

PDGF B-chain. Murine monoclonal antibody PG7-007 (provided by R. Ross and Mochida Pharmaceutical, Tokyo, Japan) has been previously characterized and its ability to specifically recognize PDGF B-chain in methacarn fixed tissue has been demonstrated [25, 26]. The specificity of the immunohistochemical reactivity of this antibody has been demonstrated in cell culture where its activity has been abolished by pre-incubation with immunizing PDGF peptide [25] and by studies by our group which tightly link increased expression of the PDGF peptide as recognized by this antibody in tissue sections with increased synthesis of mRNA encoding PDGF as detected by Northern analysis and in situ hybridization in rat glomeruli [17, 18].

PDGFRβ. Murine monoclonal antibody PR7212 has been previously characterized by Western blotting and competitive binding studies and shown to recognize the β subunit of the PDGF receptor [14, 27]. We have shown that this antibody recognizes PDGFRβ but not PDGFRα in extracts of BHK cells transfected with the human α or β subunits by Western blotting techniques. The epitope recognized by this antibody is stable in paraformaldehyde and PLP but not methacarn fixed tissues.

Smooth muscle α-actin isoform. Murine monoclonal antibody α-SM-1 (gift of G. Gabbiani, now commercially available through DAKO Corp., Carpinteria, California, USA) has been characterized by tissue immunohistochemistry and Western blotting [28] and has been previously demonstrated to recognize smooth muscle α-actin in methacarn-fixed tissues [29, 30]. We have previously demonstrated the specificity of the increased glomerular expression of α-smooth muscle actin expression detected by tissue immunohistochemistry with this antibody by concurrent Northern analysis for α-actin mRNA synthesis in isolated glomeruli obtained in a rat model of mesangiolytic injury [29].

Muscle-specific actin. Antibody HHF-35 (gift of Allen Gown, commercially available through DAKO Corp.) has been shown to recognize the four muscle specific isoforms of actin (smooth muscle α- and γ-actin, striated muscle α-actin, cardiac muscle α-actin, but not cytoplasmic β and α actins) [31] and was used as previously described [29, 30].

Smooth muscle (desmin). A murine monoclonal antibody to human desmin (DAKO Corp.) was used as previously described [32].

Endothelial cells. Rabbit anti-human Factor VIII related antigen/von Willebrand Factor (DAKO Corp.) was used as previously described [33]. Endothelial cells were also identified

by lectin binding studies using Ulex europaeus I lectin (Vector Laboratories, Burlingame, California, USA) as previously described [34, 35].

Proliferating cell nuclear antigen (PCNA). 19A2, a murine monoclonal antibody to PCNA/cyclin (Coulter Corp., Hialeah, Florida, USA) was used as previously described [36].

Immunoelectronmicroscopy

Frozen, 4% paraformaldehyde fixed kidneys were sectioned at 6 μm and sections adhered to gelatin coated slides and air dried for 30 minutes. The sections were then stained by hydrating in PBS for 15 minutes, incubated in 0.02 M periodic acid in PBS to inactivate endogenous peroxidase and then incubated with normal horse serum in PBS containing 2% BSA (Sigma, St. Louis, Missouri, USA) for 30 minutes at room temperature to block nonspecific staining. Sections were then incubated with either antibody PR7212 or α-SM-1 at 4 μg/ml in PBS/BSA overnight at 4°C, and subsequently processed using a streptavidin-biotin immunoperoxidase method with 3,3'-diaminobenzidine (DAB) as the chromogen and horse anti-mouse antisera as the secondary detecting antibody system. Sections were reacted with 1% OsO_4 in phosphate buffer for one hour 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, Pennsylvania, USA) and propylene oxide for one hour. Beem capsules were filled with PolyBed, inverted over the sections, infiltrated overnight and then polymerized at 55°C for 48 hours. The blocks were removed by heating the slide briefly and quickly snapping off the capsule. Thin, 0.1 micron sections were cut and mounted on formvar coated nickel grids and examined in a Philips 410 electron microscope.

Alternately, in efforts to improve preservation of morphologic detail, renal tissue fixed in PLP solution, was processed and embedded in LR white resin (Polysciences, Inc.). Thin sections were incubated with antibody α-SM-1 or control irrelevant monoclonal antibody overnight at 4°C, rinsed with PBS, and subsequently incubated with goat anti-mouse IgG conjugated to gold (10 nm particles) as previously described [29].

Results

Kidney tissue was obtained at gestational ages of 54 to 105 days. Kidneys from the earliest gestational ages studied (approximately 54 to 65 days) typically contain metanephric blastema, ureteric buds, interstitial tissue, several layers of immature glomeruli, and virtually no detectable structures resembling mature tubules, collecting ducts, medulla or an organized arterial or venous system. Kidneys obtained from older fetuses typically contain metanephric blastema, interstitium, ureteric buds, and glomeruli exhibiting all stages of development ranging from earliest vesicles to apparently fully differentiated, albeit immature, structures with identifiable mesangium, capillary loops, and visceral and parietal epithelial cells. A representative histologic section from such a kidney is shown in Figure 1. Also present in later kidneys are identifiable tubular segments, developing medulla, renal pelvis with urothelial lining, and distinct arterial and venous structures.

Immunohistochemistry

The immunohistochemical findings in glomeruli of this study are summarized in Table 1.

PDGF B-chain. Two developmental stage-specific patterns of PDGF B-chain expression in developing glomeruli were identified. B-chain could be first identified at the earliest stages of glomerular development, at or immediately following the vesicle stage, when the differentiating epithelium of the immature glomerulus is still in close contact with the inductive differentiating stimulus, the ureteric bud (Fig. 2A, B). In addition, the nascent parietal epithelium lining the future urinary space also expresses PDGF B-chain. In contrast, there is no detectable expression by the blastema or interstitial tissue. The ureteric bud was most often negative, but occasional staining for PDGF B-chain at the luminal surface of the ureteric bud was seen.

In later stages of glomerular development, PDGF B-chain is expressed in an arboreal pattern radiating from the hilus of the glomerular tuft, suggesting a mesangial origin (Fig. 2C). Extraglomerular structures showing expression of this protein include some immature tubules (segments distinct from ureteric structures and with features suggesting they are in contiguity with the still positive parietal epithelium, indicating proximal tubule lineage), and the superficial layers of the urothelium lining the renal pelvis and ureters.

PDGFRβ. Unlike PDGF B-chain, PDGFRβ was not detected in the glomerulus during the early stages of differentiation. Outside of the glomerulus, PDGFRβ is expressed abundantly in the metanephric blastema, and in vascular structures and interstitial cells which are seen as arcades in the most immature and superficial areas of the developing cortex (Fig. 2D). Glomerular expression of PDGFRβ occurs in later stages of development, where it localizes in a mesangial pattern (Fig. 2E, F). Occasionally, expression of PDGFRβ appeared to extend to the endothelium lining peripheral glomerular capillary walls (Fig. 2F). In adjacent maturing portions of the cortex PDGFRβ continued to be expressed by interstitial cells and arterial vessels.

Smooth muscle cell markers/mesangial cell markers. No specific markers that identify mesangial cells in tissue sections currently have been identified. However, α-smooth muscle actin, a marker for smooth muscle cells, has been shown to be an inducible marker of activated mesangial cells in disease states in adult rat and human glomeruli [29, 30]. Using an antibody to this smooth muscle marker, as well as antibodies to two additional muscle markers, pan-muscle actin and desmin, the expression of these "muscle-specific" proteins in glomerular development was studied.

Developing glomeruli show a gradient of expression of α-smooth muscle actin, ranging from absent in the earliest stages of development, to pronounced mesangial expression in more mature glomeruli (Fig. 3A, B). The results with the antibodies to both α-smooth muscle actin and muscle-specific actin were identical, with the only notable difference being somewhat stronger staining intensity obtained with the α-SM-1 antibody. The onset of smooth muscle actin expression by mesangial cells in the course of glomerular development coincided with the identification of an organized intraglomerular vasculature having contiguity with the extraglomerular interstitial vasculature (as detected by staining for Factor VIII related

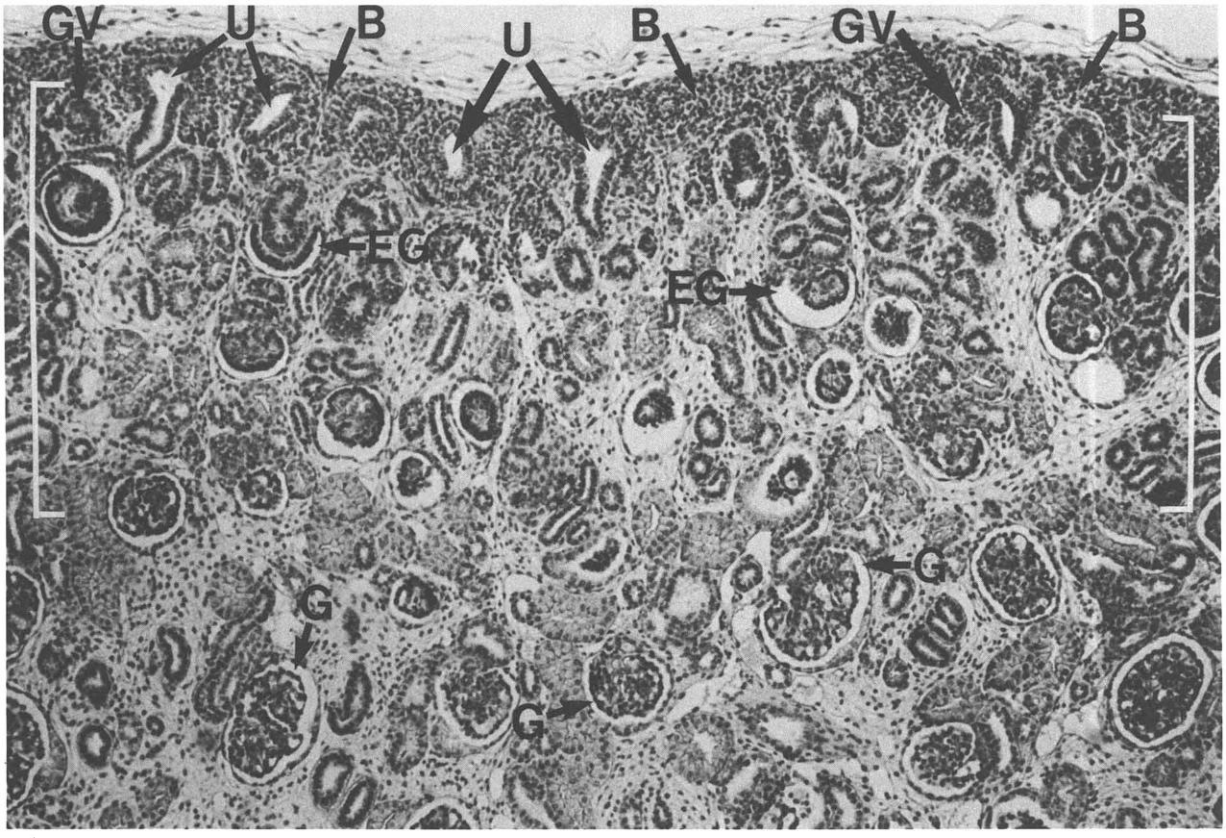

Fig. 1. *Low power micrograph of human fetal kidney (approximately 86 days gestation).* Immediately beneath the renal capsule is the undifferentiated metanephric blastema (B). Invading the blastema from below are multiple profiles of ureteric buds (U), and occasional aggregates of differentiating cells adjacent to the ureteric buds, the early glomerular vesicles (GV), can be identified. Glomeruli in zone of glomerulogenesis (within brackets) show progressive differentiation from vesicle stage, to early comma and S-stages (EG), and culminating in fully differentiated structures (G). PAS, 50×.

antigen and Ulex I lectin). There was also prominent extraglomerular expression of actin by differentiated smooth muscle cells of the arterial and arteriolar vasculature, but not by other cells comprising the interstitium.

Desmin could be identified in the smooth muscle cells comprising the media of muscular arteries present in the developing renal hilus. In contrast to the muscle specific actins, there was no localization of desmin in glomeruli at any stage of development, and no expression of this protein detectable by the antibody utilized in this study by metanephric blastema or the interstitium.

Endothelial cell markers. Factor VIII-related antigen could be identified in the aggregate of interstitial and vascular cells invaginating into the glomerular epithelium at the comma and S-shape stages of development. In more mature glomeruli, this pattern of localization evolved to a predominantly mesangial pattern similar to that of PDGF B-chain, PDGFRβ, and α-smooth muscle actin, as seen in Figure 3C. Extraglomerular staining was confined to the endothelial lining of the vasculature.

Binding of the *Ulex Europaeus* I lectin showed patterns of reactivity similar to Factor VIII-related antigen in the early stages of glomerulogenesis and in the extra-glomerular vasculature. More mature glomeruli showed discrete staining of the endothelial lining of glomerular capillaries, but did not show the apparent mesangial staining seen in the studies of Factor VIII-related antigen (Fig. 3D).

Cell proliferation. Staining for the proliferation marker PCNA was most heavily concentrated in the nephrogenic zone in the first stages of glomerular epithelial differentiation, where at times virtually every cell in differentiating vesicles, comma and S-shaped glomeruli could be shown to be actively replicating as evidenced by PCNA expression (Fig. 3E). The surrounding blastema also showed a high replicative rate. There was a decreasing gradient of PCNA expression corresponding to progressively greater glomerular maturation, in distinct contrast to the patterns of PDGFRβ and actin expression, although even the most mature glomeruli continued to exhibit some replicative activity.

Immunoelectronmicroscopy

PDGFRβ. In relatively mature glomeruli, reaction product indicative of PDGFRβ localization was confined to the membranes of cells present in mesangial locations (Fig. 4). No expression was detected in peripheral portions of glomerular capillary loops where only endothelial cells or visceral epithelial cells would be expected to be present. When endothelial cell bodies could be identified overlying the portion of mesangium adjacent to the capillary lumina, PDGFRβ could be localized to

Table 1. Phenotypic characterization of stages of glomerulogenesis in human fetal kidney

	Metanephric blastema	Vesicle	Early glomerular differentiation (comma, S-stage)	Differentiated glomerulus
PDGF B-chain	−	focal[b]	+	mesangium
PDGFRβ	+	−	−	mesangium
α-smooth muscle actin	−[a]	−	−	mesangium
Desmin	−	−	−	−
Factor VIII related antigen/ von Willebrand factor	−[a]	−	capillaries	mesangium[b] endothelium[b]
Ulex lectin	−[a]	−	capillaries	capillary endothelium

Expression of PDGF, its receptor, and differentiation markers of mesenchymal cell components of the glomerulus at the principal stages of glomerulogenesis.

Symbols are:

[a] Components of vasculature present in blastema and interstitium express these markers.

[b] It remains unresolved whether there is focal expression of PDGF in some early vesicular elements or whether these areas represent tangential sections through portions of later developing comma or S-stage vesicles; uncertainty whether Factor VIII related antigen expression is limited to endothelium or may be present in fetal mesangium is presented in the text.

PDGF—platelet-derived growth factor, PDGFRβ—platelet-derived growth factor (β subunit).

the mesangial cell borders, but not those of the endothelial cells. Because of the requirement for gentle fixation to preserve the antigenicity of the epitope recognized by the antibody to PDGFRβ, morphologic preservation of tissue was compromised to some degree, allowing confident localization of reaction product only in the anatomically well-defined structures of relatively mature glomeruli.

Muscle-specific actin (α-SM-1). The peroxidase label indicating presence of muscle-specific actin was concentrated predominantly within the cytoplasm of cells in mesangial areas identical in appearance and location to those showing staining of the cell membranes for PDGFRβ (Fig. 5). Morphologic preservation of tissues fixed in paraformaldehyde or PLP solution were both inadequate to determine whether the peroxidase or gold labels were localized to specific microfilament bundles.

Discussion

In this study, we have employed immunohistochemical techniques to study expression and distribution of PDGF B-chain, the PDGF receptor β-subunit, and markers of mesenchymal cell types in order to determine the developmental sequence and pattern of expression and provide some insight into how the PDGF system might be involved in human kidney development. All sections were from normal human fetal kidneys obtained from elective therapeutic abortions at estimated gestations of 54 to 105 days. During this developmental period, human kidneys display a gradient of differentiation from undifferentiated blastema at the outer margin of the cortex to relatively mature glomeruli in the inner portion of the cortex, as seen in Figure 1. This permits evaluations of a relatively wide span of developmental stages within a single tissue section.

Within the glomerulogenic zone, comprising a rim of cortical tissue composed of metanephric blastema, branching termini of the ureteric bud, glomeruli at the earliest stages of glomerulogenesis (vesicle, comma, and S-shaped glomeruli) and a primitive vasculature organized in arcades. Expression of PDGF B-chain was localized to differentiating cells of glomeruli vesicles and the epithelia of primitive glomeruli immediately adjacent to the ureteric bud. PDGF B-chain was not detected in the adjacent mesenchyme, blastema and blood vessels, and generally not detected in the ureteric bud. PDGFRβ, in contrast, was not detected within the primitive glomeruli but was abundantly expressed by the metanephric blastema and interstitial tissues surrounding glomeruli within the nephrogenic zone. The highest level of cell proliferation, as detected by staining for PCNA, was identified in this zone. Proliferating cells could most often be identified as epithelial cells in the earliest stages of glomerulogenesis and as metanephric blastemal cells. This suggests that PDGF released by primitive glomerular cells could be having a paracrine effect on the surrounding PDGFRβ positive primordial interstitial cells. Within the glomerulogenic zone, cells exhibiting features of muscle cell phenotype (positive for muscle actin) and endothelial phenotype (positive for F_{VIII} related antigen and Ulex lectin) were confined to the intersititial vasculature.

In more mature glomeruli located outside of the zone of glomerulogenesis, the glomerular tuft architecture assumes a more adult form and expression of PDGF B-chain, PDGFRβ, and muscle-specific actin within the glomerulus is prominent and restricted to the mesangial areas. The expression of muscle-specific actin by developing mesangial cells appeared to occur concurrently with identification of an organized intraglomerular vasculature having contiguity with the extraglomerular interstitial vasculature (as detected by staining for the markers of endothelium). These patterns of glomerular expression continued to be present throughout the period of development (to day 105) encompassed by this study. In normal adult kidneys low levels of PDGFRβ are still expressed by mesangial cells [37], but smooth muscle actin levels are greatly reduced [30], and expression of PDGF B-chain can no longer be detected by immunohistochemical techniques. The time at which fetal mesangium converts to an adult phenotype remains to be determined.

In the developing glomerulus, particularly in early stages of glomerulogenesis, the cell lineage of specific cells can be difficult to ascertain on histologic grounds alone. The histologic evidence that it is the mesangial cells which express PDGF B-chain and PDGFRβ in more mature glomeruli is supported by the congruent pattern of immunocytochemical localization of α-smooth muscle actin. By contrast, the endothelial cell-specific marker Ulex lectin stains cells with a distribution expected for glomerular endothelial cells. The immunoelectronmicroscopic results confirm, at a higher level of resolution, that α-smooth muscle actin and PDGFRβ are both expressed by cells in mesangial locations and neither are expressed by cells in endothelial locations. We therefore propose that α-smooth muscle actin is a marker for developing mesangial cells. A particularly noteworthy feature of this muscle-specific protein is that it is expressed at very low levels by mesangial cells in

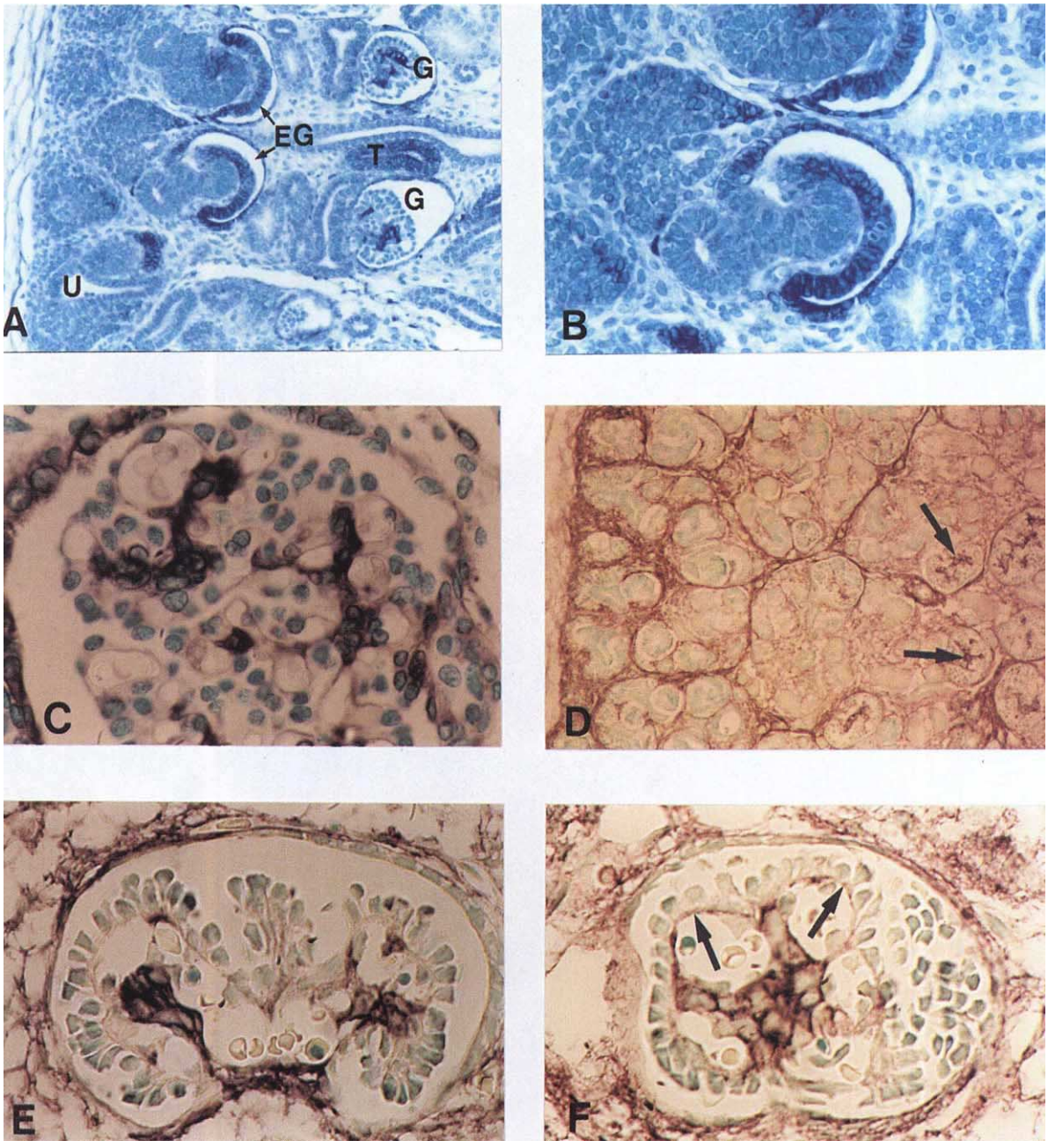

Fig. 2. *Human fetal kidney, approximately 86 days gestation.* **A.** PDGF-B chain is detected in early vesicle and comma- to S-shaped glomeruli (EG), while blastema and ureteric buds are negative. More differentiated glomeruli (G) show mesangial pattern of expression. A tubular segment (T) between a glomerulus and ureteric bud also expresses PDGF-B chain. 125×. **B.** Higher power view of early glomeruli seen in A. Staining of differentiating epithelium which will become organized into a glomerular tuft, as well as epithelial cells which will become the parietal epithelial cells lining Bowman's capsule, for PDGF-B chain is evident. 250×. **C.** Differential-d glomerular tuft showing mesangial localization of PDGF-B chain. There is uniform staining of parietal epithelial cells lining Bowman's capsule. 250×. **D.** Paraformaldehyde fixed tissue, from same kidney as shown in A through C, stained for PDGFRβ. There is localization of PDGFRβ to blastema, vascular structures (present in arcades of interstitial tissue), interstitial cells, and mesangial areas (arrows) of more mature glomeruli. 60×. **E.** Differentiated glomerulus stained for PDGFRβ. Staining is confined to mesangial areas and extraglomerular interstitium. 250×. **F.** Differentiated glomerulus stained for PDGFRβ. In addition to mesangial expression of this protein, occasional staining seemed to extend to glomerular capillary endothelium (arrows). 250×.

normal mature human glomeruli, and its expression is dramatically upregulated in rat [29] and human [30] glomeruli in response to injury.

Nonetheless, there are some limitations in our ability to definitively state that PDGF and PDGF-R are expressed only by mesangial cells. Immunostaining with antibody to F_{VIII}-related antigen, an antigen that is generally considered to be expressed exclusively by endothelial cells, reveals a pattern of localization in fetal glomeruli in later stages of development that can be indistinguishable from that of α-smooth muscle actin, PDGF,

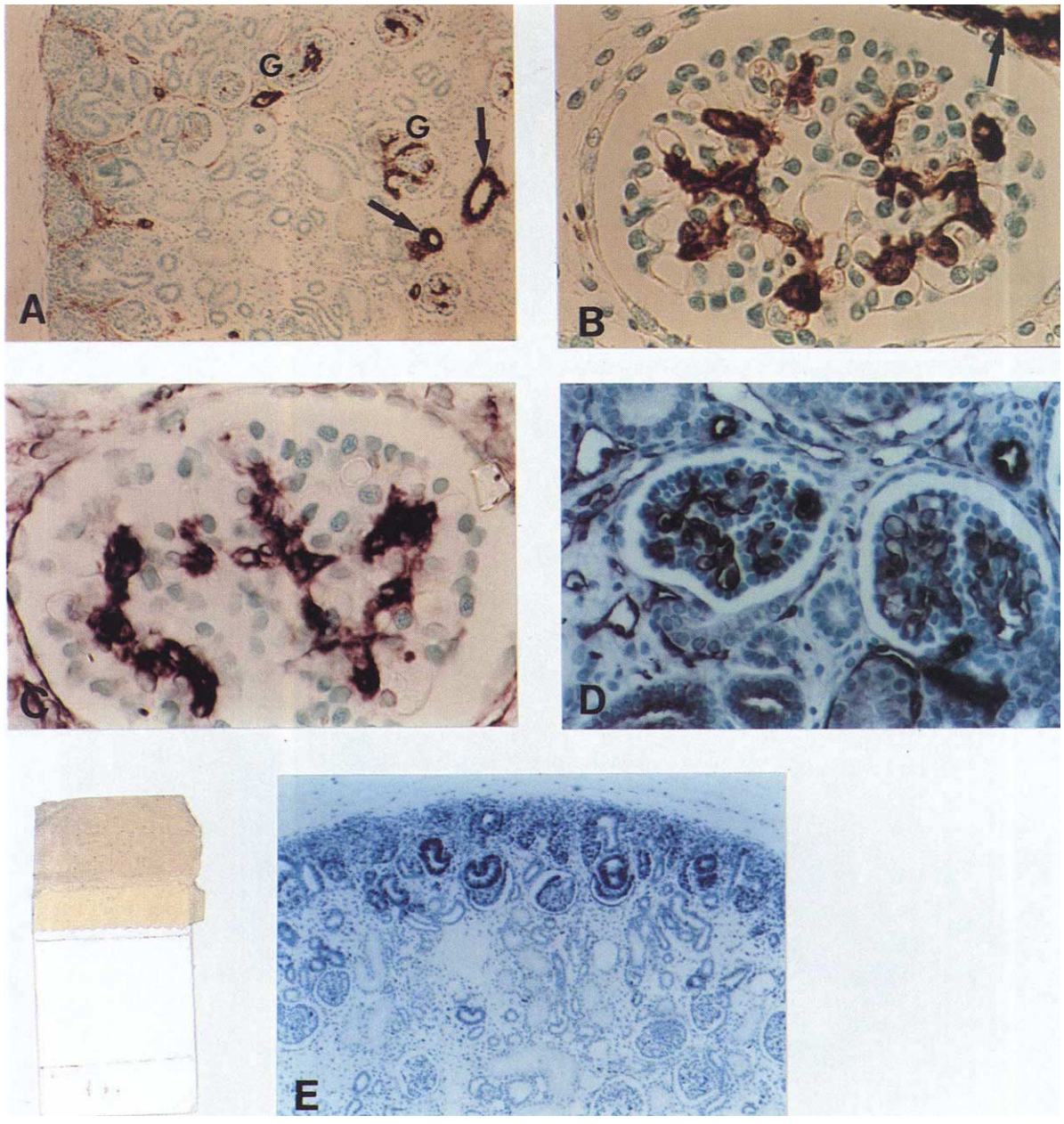

Fig. 3. *Same fetal kidney illustrated in Figure 2.* **A.** Expression of α-smooth muscle actin. There is localization in vessels coursing through the blastema, arterial and arteriolar smooth muscle cells (arrows) and in the mesangium in later stages of glomerular development (G). 60×. **B.** Higher power view of α-smooth muscle actin expression in mesangium and in adjacent arteriolar smooth muscle cells (arrow). 250×. **C.** Expression of Factor VIII related antigen also appears to have a mesangial rather than pure endothelial pattern. These findings present a paradox, suggesting in the fetus that there are glomerular (apparently mesangial) cells with features of both endothelial and smooth muscle derivation. 250×. **D.** Binding of Ulex I lectin contrasts with that of Factor VIII related antigen in clearly exhibiting an endothelial pattern of reactivity including glomerular capillary walls and interstitial vessels and capillaries. 125×. **E.** Staining for proliferating cell nuclear antigen (PCNA), indicative of replicating cells, is concentrated in blastema and in epithelial cells comprising early glomeruli in zone of glomerulogenesis. Positively stained cells are less numerous but still present in more differentiated glomeruli as well as developing tubules and interstitium. 60×.

and PDGF-R. There are at least two possible explanations for this. (1) The first possibility is that mesangial cells originate from a mesenchymal precursor with some properties of both endothelial cells (F_{VIII} related antigen) and smooth muscle cells (α-smooth muscle actin) and that expression of F_{VIII} is lost relatively late in mesangial cell differentiation. (2) A second possibility is that the apparent F_{VIII} staining of mesangial cells is an artifact resulting from a close apposition of endothelial cells to the mesangium, with regional concentration of F_{VIII} related antigen in those parts of the endothelial cells (nucleus

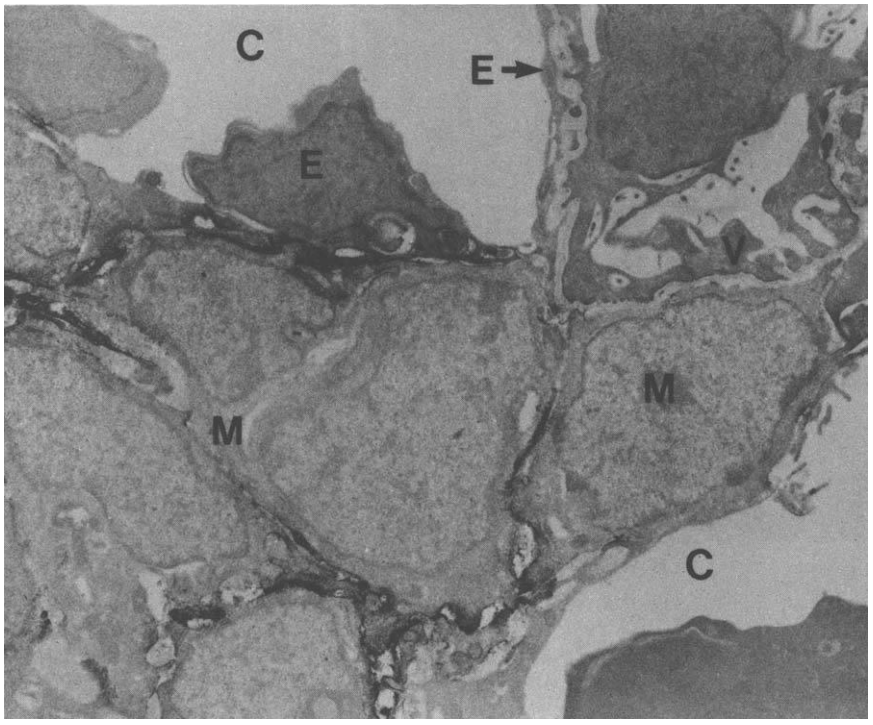

Fig. 4. *Ultrastructural immunolocalization of PDGFRβ.* Black peroxidase reaction product is confined to the cell membranes of cells within mesangial areas (M). Visceral epithelial cells (V), and capillary endothelium (E) are not reactive. C, capillary lumen. 8800×.

and large parts of the cell body) adjacent to the mesangial cells, rather than distributed in the thin layers of fenestrated endothelium which line the peripheral parts of the glomerular capillary wall. Such a distribution would explain why the other endothelial cell marker, Ulex europaeus I lectin, did not show a mesangial staining pattern, since Ulex binds to an αL-fucose carbohydrate moiety distributed diffusely over the surface of the endothelial cell [34] rather than to a cytoplasmic component. Resolution of this issue may come when we or others develop the ability to successfully combine markers for F_{VIII} related antigen, actin, and PDGF-R in single sections studied by immunoelectron microscopy.

The spatial and chronological patterns of expression of PDGF B-chain and PDGFRβ in the developing metanephric kidney suggest that this growth factor/receptor interaction might have multiple roles in the process of glomerular differentiation and maturation. At the earliest stages of glomerulogenesis, PDGF B-chain is expressed by the vesicle while the PDGFRβ is expressed only outside the vesicle by blastemal and connective tissue elements. This suggests a possible paracrine interaction within the kidney, in which PDGF from the vesicle may modulate the processes of glomerular induction or begin to stimulate migration of receptor-positive cells from the vascular/connective tissue into the glomerular cleft where they develop into the mesangial stalk. There is growing evidence that PDGF can act as such a migratory chemotactic factor for fibroblasts [38], smooth muscle cells [39–40] and even mature mesangial cells [41]. Once the mesangial-like cells have entered the glomerulus they continue to express PDGFRβ but now also express PDGF B-chain. This might provide an autocrine stimulus for further mesangial cell proliferation, migration, and/or modulation. Eventually, expression of both PDGF B-chain and PDGFRβ is downregulated, further proliferation virtually ceases, and the glomerulus achieves its mature/adult phenotype.

The autocrine phase of development may be reactivated in adults under pathological conditions in which mesangial cell proliferation is reactivated. We have recently provided evidence that induced expression of PDGF B-chain and PDGFRβ may mediate mesangial cell proliferation and participate in reconstitution of the mesangium following antibody-induced mesangiolytic injury in the rat [17, 18]. In man, there is some limited evidence that upregulation of PDGF-R may occur in the course of some forms of glomerulonephritis [19] as well as evidence that PDGF may localize to the mesangium in some forms of mesangial proliferative glomerulonephritis [20, 42]. Furthermore, it has been demonstrated that cultured mesangial cells stimulated by a variety of mediator substances known to participate in renal injury will produce PDGF [16, 43, 44]. It is particularly noteworthy that in both the rat [17, 29] and humans [30], this apparent upregulation of the PDGF/PDGF receptor system in disease states is associated with phenotypic modulation by mesangial cells to increase expression of muscle-specific actin, similar to that identified in fetal glomeruli. We believe these lines of evidence demonstrate that the PDGF/PDGF receptor system mediates at least in part the proliferative and reparative phases of mesangial injury in experimental animals and in humans. The data presented in this study therefore suggests that this response to injury recapitulates a normal process of mesangial development that occurs in utero.

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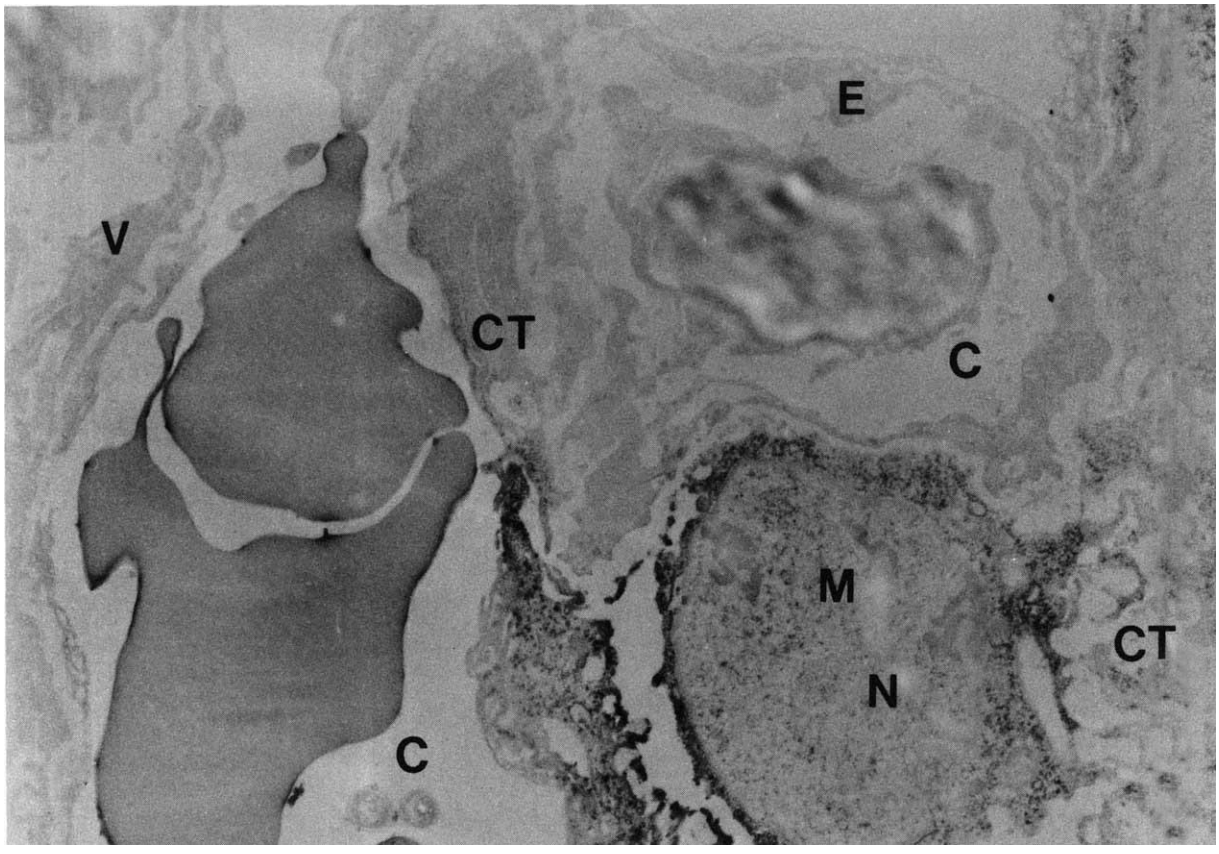

Fig. 5. *Ultrastructural immunolocalization of α-smooth muscle actin.* Black peroxidase reaction product is confined to cells in mesangial areas (M). Visceral epithelial cells (V), endothelial cells (E), mesangial cell nuclei (N) and connective tissue matrix (CT) in mesangium and glomerular basement membranes are not reactive. C, capillary lumen. 8300×.

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