A serum-free in vitro model of renal microvessel development

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1Renal-Electrolyte and Hypertension Division, Department of Medicine, Philadelphia Veterans Affairs Medical Center and University of Pennsylvania School of Medicine; 2Center for Oral Health Research, University of Pennsylvania School of Dental Medicine, Philadelphia 19104; and 3Department of Pathology, Allegheny University of the Health Sciences, Philadelphia, Pennsylvania 19102

Antes, Lisa M., Monica M. Villar, Sylvia Decker, Roberto F. Nicosia, and Dean A. Kujubu. A serum-free in vitro model of renal microvessel development. Am. J. Physiol. 274 (Renal Physiol. 43): F1150–F1160, 1998.—The differentiation and organization of the embryonic renal vasculature is a crucial event in renal development. To study this process, we developed a serum-free in vitro model of renal microvessel development. Mouse embryonic kidney explants, when embedded specifically in type I collagen, demonstrated outgrowth of microvascular structures when stimulated by the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA, 10–50 ng/ml). Other polypeptide growth factors stimulated little, if any, microvessel outgrowth from the explants. Similar outgrowths were not observed when other embryonic tissue explants were used. The number of microvessels observed depended on the gestational age of the explants. We hypothesize that TPA induces the in situ differentiation of metanephric mesenchymal cells into endothelial cell precursors and that specific matrix proteins and cell-matrix interactions are necessary for the organization of these precursors into microvessels. Our model will allow us to examine in detail the responsiveness of metanephric kidney cells to both growth factors and extracellular matrix molecules and to understand how they influence renal endothelial cell differentiation.

Angiogenesis; vasculogenesis; renal endothelium

The kidney’s complex microscopic anatomy, with discrete physiological functions occurring in specific regions of the organ, necessitates a supporting vasculature with equally complex features. Unfortunately, little is known about what regulates renal vascular development. The origin of the renal endothelial cells remains uncertain. Although metanephric mesenchyme, when cocultured with ureteric bud in vitro, differentiates into an avascular organ possessing tubular and glomerular structures, the latter lacks both endothelial and mesangial cells (2). Vascularized glomeruli are observed, however, when mouse metanephric kidney rudiments are transplanted onto the anterior chamber of mice transgenic for β-galactosidase, only rare host endothelial cells are observed in the vascularized glomeruli (13, 25). The majority of metanephric endothelial cells are of donor origin. These results suggest that, although invasion of host endothelial cells into the transplant can occur, in general, vascular precursor cells within the metanephric mesenchyme differentiate in situ to form the renal microcirculation (vasculogenesis) (1, 13, 25).

Resolution of this controversy depends on identifying the important morphogenic cues provided to the developing renal endothelial cells and on understanding the cellular responses to them. Unfortunately, unlike renal epithelial differentiation, where in vitro models are available, there are no convenient systems by which to study renal vascular development in a controlled fashion. Neither the CAM nor the anterior-chamber angiogenesis assays allows one to independently manipulate both exogenous growth factors and extracellular matrix molecules. Angiogenesis assays using isolated endothelial cell lines do not mimic the complex microenvironment of the developing kidney and the interplay of the multiple cell types present. Moreover, the “capillary tubes” derived from endothelial cell lines lack periendothelial cells (pericytes), which are thought to play important regulatory roles in capillary formation and maintenance in vivo (6). The development of alternative in vitro assays would greatly aid in the delineation of important factors involved in the formation of the renal microcirculation.

Our objective was to identify and characterize soluble angiogenic factors elaborated from the metanephric kidney that might be involved in renal microvascular development. To accomplish this, we designed a serum-free in vitro system in which mouse metanephric explants were cocultured with adult aortic rings in a collagen matrix support. We reasoned that soluble angiogenic factors produced by the metanephric kidney would stimulate the development of microvascular structures from the aortic macrovascular endothelium. Instead, we found that metanephric kidney explants themselves were capable of demonstrating microvessel outgrowth under specific conditions. Our model will allow us to systematically explore the factors involved in the differentiation of renal microvascular endothelium from metanephric mesenchymal cells.
MATERIALS AND METHODS

Reagents. A 1× Ham's F-12 nutrient mixture (HAM) with L-glutamine and 10× MEM with Earle's salts were purchased from Life Technologies (Gaithersburg, MD). Endothelial cell basal medium (EBM) was from Clonetics (San Diego, CA). Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and Matrigel were obtained from Collaborative Biomedical Products (Bedford, MA). Platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-α (TGF-α) and -β (TGF-β) were purchased from Life Technologies. Formalin and materials used for histological sample preparation were purchased from Fisher Scientific (Malvern, PA). 12-O-tetradecanoylphorbol 13-acetate (TPA) and agarose type I-A:low electroendosmosis were purchased from Sigma (St. Louis, MO). Purified rat anti-mouse CD34 antibody was obtained from Pharmingen (San Diego, CA), and purified rabbit anti-human von Willebrand factor antibody was purchased from Dako (Carpinteria, CA). Avidin-biotin peroxidase complex (ABC) reagents for immunohistochemistry were obtained from Vector Laboratories (Burlingame, CA). Signal was enhanced with biotinyl tyramide purchased from Becton-Dickinson Laboratories (Lincoln Park, NJ).

Cultures of metanephric explants. Intestinal collagen was prepared from the tails of Sprague-Dawley and Wistar rats (ACE Animals, Boyertown, PA), according to the method of Elsdale and Bard (8). Collagen gels were prepared in 1.5% agarose rings from a 1–3 mg/ml solution of the interstitial collagen (24). Mature CD1 mice obtained from Charles River Laboratories (Wilmington, MA) were mated, and the pregnant females were anesthetized and then killed by cervical dislocation. Embryos from days 12–20 (E12–20) were retrieved by cesarean section. Kidneys were microdissected free using an Olympus SZ40 dissecting microscope and were washed in serum-free medium (MEM-F12, supplemented with penicillin-streptomycin and glutamine). Embryonic kidneys were embedded in the rat tail collagen and grown in culture for 7 days in a 5% CO₂ incubator. The serum-free culture medium (EBM, supplemented with 1% penicillin-streptomycin) was replenished three times per week, and microvessel outgrowths were observed and quantitated by two independent observers using an Olympus CK2 inverted light microscope, as described by Nicosia and Ottinetti (24). Microvessels were distinguished from fibroblastic outgrowths by the presence of distinct lumens surrounded by flattened cells. Quantitative results from the two observers were consistently within 10% of each other.

Electron microscopic studies. To confirm histologically that these outgrowths were indeed of endothelial origin, the specimens were prepared for electron microscopy. They were fixed in 4% paraformaldehyde and 5% glutaraldehyde and postfixed in osmium tetroxide. After dehydration through a series of graded alcohols, they were slowly infiltrated with Epon (epoxy resin) and polymerized. Ultrathin sections were cut using a diamond knife, contrasted with uranyl acetate and lead citrate, and evaluated in a JEOL 100CX II transmission electron microscope operated at 80 kV.

Lectin staining and immunohistochemistry. For lectin staining, collagen-embedded, TPA-stimulated embryonic renal explants were Formalin fixed, dehydrated through a series of graded alcohols, embedded in paraffin, and sectioned using a microtome. Thirty-micrometer sections were obtained to ensure that microvessels were present in each section. Specimens were then incubated with either biotin-conjugated Bandeiraea simplicifolia I-B₄ (BSI-B₄) (20 µg/ml) or biotinylated BSI-B₄ (Sigma) in 0.2 M galactose for between 2 and 3 h. The sections were washed serially in PBS with 10 mM CaCl₂ and 1 mM MgCl₂, then incubated with ABC reagent (Vector). To enhance the signal, specimens were incubated in biotinyl tyramide, according to the manufacturer's instructions (DuPont-NEN), prior to adding the diaminobenzidine indicator reagent (Vector).

For immunohistochemical studies, similarly prepared sections were incubated for 60 min, using a purified rat anti-mouse CD34 antibody (10 µg/ml), washed serially with PBS, and then incubated with a biotin-conjugated mouse anti-rat IgG antibody (dilution 1:250). After serial washing with PBS, the signal was enhanced, using biotinyl tyramide prior to incubation with the diaminobenzidine indicator reagent. For von Willebrand factor staining, paraffin-embedded sections were incubated with 0.1% proteinase K for 10 min prior to incubation with a purified rabbit anti-human von Willebrand factor antibody (dilution, 1:50). After serial washes, the specimens were incubated sequentially with a biotinylated goat anti-rabbit IgG antibody (dilution, 1:200), ABC reagent, and diaminobenzidine indicator reagent.

RESULTS

Outgrowth of microvessel-like structures from metanephric kidney explants. Our initial objective was to determine whether metanephric kidney explants elaborate soluble angiogenic factors capable of stimulating endothelial cell growth and differentiation. To accomplish this, we used a modification of a serum-free in vitro angiogenesis assay described by Nicosia et al. (23). They had previously shown that rat aortic rings demonstrate striking microvessel outgrowth when embedded in type I collagen and stimulated with angiogenic growth factors. When mouse metanephric kidney explants were cocultured with aortic rings in the presence of type I collagen, we observed cellular outgrowths arising both from the aorta and the kidney. None of the outgrowths, however, had the appearance suggestive of microvessels. To determine whether the addition of exogenous angiogenic factors could stimulate microvessel formation, we incubated the coculture preparations with TPA, a potent inducer of angiogenesis. After TPA stimulation, we observed numerous cellular outgrowths arising from both the aorta and the kidney as before, but to our surprise, the outgrowths were particularly exuberant from the kidney (Fig. 1). Although many outgrowing cells had a fibroblastic phenotype with long cellular projections, others had the appearance suggestive of vascular structures, i.e., highly attenuated cells surrounding a centralized lumen. Of interest, we did not observe microvessel-like outgrowths from the TPA-stimulated mouse aortic rings as we had expected. To determine whether metanephric kidney explants in the absence of aortic rings were capable of demonstrating these outgrowths, the microdissected explants were embedded in type I collagen and cultured in the presence and absence of TPA. TPA stimulated the outgrowth of microvessel-like structures from the metanephric explants in the absence of aortic rings (Fig. 2). To determine whether microvessel-like structures arise only from the surface of the metanephric kidney, surgically transected explants were embedded in collagen and stimulated with TPA. We
observed neither quantitative nor qualitative differences in the microvessel-like outgrowths originating from the surface of the kidney, compared with the transected edge (data not shown). To determine whether other embryonic tissues are similarly capable of demonstrating microvessel outgrowth with TPA stimulation, we used embryonic lung, intestine, liver, heart, and placenta explants in our system. Although TPA-stimulated embryonic lung, liver, and intestine explants demonstrated occasional microvessel outgrowth, the response was quantitatively much less than that seen with the embryonic kidney (Fig. 3). Microvessel outgrowth was not observed from TPA-stimulated, collagen-embedded explants obtained from either embryonic heart or adult placenta.

Vascular nature of the cellular outgrowths. To better visualize the microvessel-like structures, collagen-embedded, TPA-stimulated metanephric explants were prepared for electron microscopic analysis. We observed that the outgrowing structures were composed of polarized flattened cells with protruding nuclei encircling a central lumen (Fig. 4). The cells possessed tight junctions, lacked brush borders, and contained pinocytic vesicles. Some microvessels were partially surrounded by periendothelial cells possessing a characteristic electron-dense cytoplasm. To further confirm the endothelial cell origin of the structures, fixed, TPA-treated, collagen-embedded explant sections were stained with Bandeiraea simplicifolia I-B4 (BSI-B4), a lectin that preferentially binds to mouse endothelial cells (13, 17, 19). We observed that the lumen-bearing cellular outgrowths bound biotinylated BSI-B4 and that the binding was inhibited by galactose, a competitive inhibitor (Fig. 5). Moreover, the cellular processes stained positively for CD34, a marker of microvascular endothelial cells (Fig. 6) (15) but were negative for von Willibrand factor, a marker of mature macrovascular endothelial cells (data not shown) (5).

To determine whether the microvascular structures arise from preexisting vessels within the metanephric parenchyma, we examined additional sections of the explants both by light and electron microscopy. We
were unable to observe any clear continuities between vessels within the parenchyma and the outgrowing microvascular structures. Instead, the microvessels appeared to arise from mesenchymal cells at the surface of the explants (Fig. 7). Many of the cells within the parenchyma, however, had pyknotic nuclei, and the architecture of the metanephric parenchyma itself was difficult to interpret. Although glomerular structures were clearly visible within the explants, architectural distortion precluded us from determining whether vascularization of the glomeruli occurred under our culture conditions. Unlike the culture conditions established by Grobstein (11), explants cultured under our serum-free conditions did not continue to increase in size.

Effects of TPA and growth factors on microvessel outgrowth. To determine whether other growth factors that have been suggested to play roles in metanephric development and vascularization in vivo can stimulate microvessel outgrowth in our system, we prepared metanephric explants as before and stimulated them with VEGF, PDGF-BB, bFGF, and TGF-α and -β, all of which are reported to possess angiogenic activity in various systems (Fig. 8). Of the growth factors tested, only TGF-α was capable of stimulating more than a few microvessels from the explants in the doses used. Several of the growth factors tested elicited other notable biological responses, however. Both bFGF and TGF-α stimulated a dense outgrowth of fibroblastic cells, and high doses of TGF-β induced striking disintegration of the explants. Although TPA is not a growth factor in the strictest sense, it was clearly the most potent stimulator of microvessel outgrowth of the agents we tested. To determine whether the number of outgrowths was dependent on the dose of TPA used, collagen-embedded explants were treated with various doses of TPA for 7 days (Fig. 9). We observed a sharp dose dependence of microvessel outgrowth, with concentrations of 10–50 ng/ml demonstrating maximal microvessel outgrowth. The number of microvessels observed decreased when concentrations higher than 50 ng/ml were used. Moreover, when the higher doses of TPA were used, the explants demonstrated marked cortical disintegration. This was often accompanied by degradation of the surrounding collagen matrix, allowing the previously embedded explant to float free.

Effects of changes in extracellular matrix on microvessel outgrowth. Embryonic kidneys, when cultured solely in TPA-containing media without a supportive collagen matrix, did not demonstrate microvessel outgrowth,

Fig. 3. Microvessel-like outgrowth from embryonic tissue explants. E16 mouse kidneys, intestine, lung, liver, heart, and placenta were embedded in type I collagen and treated with TPA (25 ng/ml) for 7 days. Microvessel-like structures were quantitated after 7 days in culture.
underscoring the importance of matrix molecules in this process (data not shown). Increasing the concentration of the collagen matrix to 2 mg/ml resulted in broader and more arborized microvessels than that seen with 1 mg/ml; however, there were no quantitative differences in microvessel outgrowths observed. In preliminary experiments using collagen at 3 mg/ml, we continued to observe TPA-stimulated microvessel development from the earlier gestation kidneys (i.e., E13 and E14). In contrast, when Matrigel, a commercially available basement membrane-type matrix composed of a mixture of type IV collagen, laminin, and entactin was used in our model, a dense outgrowth of mesenchymal cells was observed, but no microvessels were detected (Fig. 10). TPA facilitated the palisading of these fibroblastic cells. Our findings indicate that type I collagen, but not Matrigel, provides the cell-matrix interactions permissive for TPA-induced microvessel outgrowth. These cell-matrix interactions alone, however, are not sufficient to promote microvessel outgrowth in our system.

Effects of gestational age on microvessel outgrowth. To determine whether the gestational age of the explant influenced the number of microvessels we observed following TPA treatment, we obtained metanephric kidneys from embryos at different stages of gestation. Although there is some variability between experiments, we observed that maximal microvessel outgrowth was obtained with kidneys from embryos at days 15–17 of gestation (Fig. 11). Of interest, very few microvessels were observed from kidneys obtained from neonatal mice, despite the fact that murine nephron development proceeds for several days postnatally. No TPA-stimulated microvessels were observed when wedges of adult kidney tissue were used in our preparations.

Fig. 4. Ultrastructural examination of the microvessel-like structures. A: microvessel-like structure arising from a collagen-embedded TPA-treated E14 mouse kidney was sectioned and examined ultrastructurally. B: longitudinal section of microvessel-like structure. Bars represent 1 µm. Photographs are representative of sections obtained from ~6 lumen-bearing structures.
Our observation that mouse metanephric explants develop microvessel outgrowths when embedded in type I collagen and stimulated with TPA provides us with a unique opportunity to identify factors critically important in regulating renal endothelial cell growth and differentiation. Unlike studies that simply document the expression of specific molecules in the developing kidney, our model allows us to probe the responsiveness of metanephric kidney cells to both growth factors and extracellular matrix molecules independently under serum-free conditions. As with any in vitro model, however, ours cannot claim to recapitulate all aspects of renal microvascular development occurring in vivo. Although our culture conditions favor the growth of microvessels, they fail to sustain the continued differentiation of epithelial structures. Because of that, important interactions between the developing endothelial cell network and neighboring tubular cells in the developing kidney cannot be examined in our system. The microvascular structures that do develop in our culture system, however, arise from metanephric tissue, have a characteristic morphology, possess pericytes, and stain for markers of microvascular endothelial cells. By exploiting features of this model, we ultimately hope to identify factors responsible for the regional phenotypic and functional differences among endothelial cells within the kidney.

The ability of TPA to stimulate angiogenesis in vivo is well known (22, 29). Montesano and Orci (21) observed that, following in vitro TPA treatment, endothelial cells invade an underlying collagen matrix and form capillary-like structures in the absence of ongoing DNA synthesis. In our system, TPA is a potent stimulator of microvessel outgrowth from metanephric explants. Our preliminary experiments suggest that TPA exerts its effect by activating protein kinase C (PKC), since a selective PKC inhibitor, bis-indolylmaleimide, could completely inhibit TPA-induced microvessel outgrowth.
Given that microvessel outgrowth was not significantly stimulated by PDGF-BB, bFGF, VEGF, and TGF-β, it is unlikely that the effect of TPA is due to its ability to induce the local production of these growth factors. It is possible, however, that there might exist other novel embryonic growth factors, whose action is mimicked by TPA in our system. One logical starting point is to determine whether tumor-induced angiogenic factors might similarly stimulate microvessel outgrowth in our system, since many tumors are known to express embryonic antigens. In preliminary experiments, we observed that conditioned media from two different tumor cell lines were capable of stimulating microvessel outgrowth. Our serum-free model will be instrumental in helping us to isolate and characterize novel angiogenic factors.

We were somewhat surprised that potent angiogenic factors, such as VEGF and bFGF, were not capable of stimulating significant microvessel outgrowth in our system. After failing to see more than just a few microvessels in response to these growth factors at a variety of concentrations, it became evident to us that the superficial mesenchymal cells of the explants, i.e., those exposed to the dual stimuli of TPA and type I collagen, were not typical of endothelial cells. We speculate that the microvessels we observe are the cumulative result of two processes: 1) the differentiation of superficial mesenchymal cells into angioblasts and 2) the assembly of angioblasts into mature endothelium. We hypothesize that, while VEGF and bFGF are capable of inducing the latter, they are unable to stimulate the differentiation of metanephric mesenchymal cells into angioblasts. In contrast, TPA is capable of stimulating both processes. Experiments are underway to determine whether the microvessel-like structures, once formed, elongate in response to either VEGF or bFGF.

Type I collagen, the predominant matrix molecule present in the metanephric kidney (3), is permissive for microvessel outgrowth in our system. In contrast, Matrigel, a commercial product composed primarily of type IV collagen, laminin, and entactin, is unable to support microvessel outgrowth. Matrigel also contains a number of growth factors and matrix molecules capable of supporting the tubular morphogenesis of renal epithelial cells (26, 27). It is possible that the growth factors and minor components in Matrigel exert inhibitory effects on the outgrowth of microvessels from the embryonic explants. Alternatively, the cells capable of forming microvessels may possess specific cell surface receptors for type I collagen molecules and not the matrix components in Matrigel. The specific receptor-ligand interactions may then initiate a cellular program resulting in the proliferation, adhesion, and...
motility of endothelial cells culminating in microvessel formation. Part of the program may involve the expression of novel cell surface molecules. For example, using the CAM angiogenesis system, Cheresh et al. (4, 10) observed that both $\alpha_v\beta_3$- and $\alpha_v\beta_5$-integrins are critically important mediators of new vessel formation.

Although the temporal and spatial expression of $\alpha_v$-integrins in the developing kidney has been examined

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**Fig. 9.** Microvessel outgrowth in response to TPA. Microvessel outgrowth from collagen-embedded, E17 kidneys was quantitated following incubation with TPA for 7 days. Graph demonstrates a representative time course and dose-response relationship of microvessel outgrowth to TPA.

**Fig. 10.** Embryonic kidney embedded in Matrigel. E15 kidneys were embedded in Matrigel and incubated in the absence (A) and presence (B) of 25 ng/ml TPA.
(30), factors regulating their expression are not known. If TPA induces the expression of the $\alpha_v$-integrins in our system, we can explore their regulation in more detail.

In addition to stimulating microvessel outgrowth from the metanephric kidney, TPA enhances cortical disintegration of the explant and degradation of the surrounding collagen matrix. Although the migration of mesenchymal cells into the collagen matrix may in part be responsible for our observation, it is likely that TPA-stimulated mesenchymal cells elaborate metalloproteinases capable of disrupting the integrity of the metanephric parenchyma and supporting matrix. It is well known from other models of angiogenesis that localized collagenase release is an essential early step in the formation of new vessels (9, 12). With 1 mg/ml collagen preparations, we observe that microvessel outgrowth quantitatively diminished in the later gestation explants and rarely occurred in postnatal kidneys, despite continued nephron development and a progressive increase in explant size and surface area. TPA-stimulated collagenase expression in the late gestation and postnatal kidneys may be insufficient to promote exuberant microvessel outgrowth. Interestingly, the earlier gestation metanephric kidneys, i.e., E13–E14, may be capable of releasing higher local levels of collagenase than later gestation explants, enabling microvessel outgrowth to occur even in 3 mg/ml collagen preparations. With the availability of antisera and probes to collagenases, as well as specific metalloproteinase inhibitors, these hypotheses are testable using our model.

It is interesting that, of the embryonic tissues tested, only the kidney was capable of producing more than just a few microvessels under our conditions. Montesano et al. (20) observed microvessel outgrowth from explants of rat embryonic muscle and adipose tissue, suggesting that the phenomenon we observe is not limited to the kidney. To determine whether microvessels can arise from either an embryonic organ with functional endothelium or an adult organ characterized by intense vascularity, we tested both embryonic heart and adult placenta under our culture conditions. Neither of the organs was capable of developing microvessels in our hands. This suggested to us that the microvessels we observe from our kidney explants arise not from preexisting endothelial cells (angiogenesis) but rather from the differentiation of metanephric cells into an endothelial phenotype. Alternatively, resident metanephric endothelial cells may be particularly sensitive to the angiogenic effects of both PKC and integrin activators compared with endothelia from other embryonic and adult tissues.

Conditions that favor the development of the microvessel outgrowths are strikingly similar to those that facilitate branching morphogenesis of tubular structures in epithelial cells, namely PKC activators and collagen (26, 27). It is interesting that we did not observe the outgrowth of epithelial structures from a tissue that ultimately becomes essentially epithelial in nature. Our culture conditions appear to favor the growth of endothelial cells but fail to sustain epithelial cells. One reason for this might be due to the medium we use, which was initially designed to support human microvascular endothelial cell growth (16). In that medium, epithelial cells may require additional serum factors, which we do not provide, to prevent apoptosis. Alternatively, the collagen preparation we use may be subtly different from commercially available products capable of supporting branching morphogenesis of epithelial structures. In pilot experiments, we found that commercially available collagen stimulated a marked fibroblastic outgrowth from metanephric explants, even in the absence of TPA. This differed strikingly from what we observed when we used our collagen preparation. We believe that our specific culture conditions select for the viability of specific mesenchymal cell populations in favor of other cell types and that TPA promotes the full development of the endothelial phenotype by those cells.

Does our model provide clues as to how renal vascular development occurs in vivo, by angiogenesis or vasculogenesis? Because the process of vascularization is well underway within the metanephric kidneys we used in our preparations, we cannot directly address
this question. Results from our in vitro model, however, allow us to make some cautious conclusions. The microvessels we observe are clearly of renal origin and appear to derive from the surface of the kidney. We have not observed continuities among intraparenchymal vessels and those extending out from the organ. We did not observe either quantitative or qualitative differences in microvessels arising from the transected edge of the embryonic kidney compared with the surface. Had the microvessels originated from preexisting vascular structures, one would have expected to see many more microvessels originating from the transected surface of the organ, since vessel development is more extensive in the central portion of the kidney compared with the cortical regions. Microvessel development was rare or nonexistent from other embryonic and adult tissues, even those with functional endothelia. Moreover, microvessel development occurred maximally within a discrete gestational window, despite murine nephron development proceeding postnatally. In summary, our findings suggest that metanephric mesenchymal cells demonstrate remarkable plasticity; under appropriate conditions, they are capable of differentiating into capillary endothelial cells, consistent with the notion of vasculogenesis. Future experiments will be aimed at determining whether this cellular plasticity is operational in vivo.

Although the majority of the renal vessels remain within the kidney parenchyma proper, some blood vessels do extend beyond the surface of the kidney in the adult animal. One situation where the capsular vessels with their perforating and nutrient branches become prominent is in conditions where blood flow through the main renal arteries is hindered, i.e., renal artery stenosis. Although part of the collateral blood flow results from the distension of poorly perfused preexisting vascular channels, part is clearly due to the formation of new vessels, demonstrating that the adult organ is capable of angiogenesis. In contrast, the regenerative capacity of the highly specialized glomerular endothelium following injury is less clear. Although, in some animal models of glomerulonephritis, glomerular endothelial regeneration does occur, in many cases, glomerular injury proceeds into sclerosis. In those situations, either the glomerular microenvironment may be unsuitable for endothelial differentiation or glomerular endothelial precursor cells may be lacking in those glomeruli destined for sclerosis. The answers to these questions await the development of cellular markers for mature glomerular and renal vascular endothelial cells and their respective precursors.

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