American Journal of Pathology, Vol. 147, No. 3, September 1995

Developmental Expression of the Nephritogenic Antigen of Monoclonal Antibody 5-1-6

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The biogenesis of p51, the target of nephritogenic monoclonal antibody 5-1-6, was studied in the developing glomerulus by immunolocalization and metabolic labeling. The localization of p51 was compared with that of ZO-1, a component of the cytoplasmic face of the epithelial slit diaphragm, and with that of podocalyxin, an apical marker of the podocyte. p51 first became faintly, but clearly, detectable on the basal and lateral sides of the developing podocytes at the S-shaped body stage. Staining intensity increased with further maturation and was restricted to the visceral epithelial cells. On immunoelectron microscopy, the antigen was seen along the basal and lateral surfaces below occluding junctions at the early capillary loop stage and later, with the interdigitation of foot processes, became concentrated in the slit pores. At no stage was p51 seen on the apical surface. p51 and ZO-1 were closely localized in the mature glomerulus but arrived at their final positions from opposite directions. p51 was on basal and podocalyxin was on apical sides of the glomerular epithelium from the S-shaped body stage onwards. Metabolic labeling studies showed that p51 is actively synthesized during initial glomerular development and that the rate of synthesis declines substantially with maturation. We conclude that p51 is primarily synthesized during initial glomerular development, becomes concentrated in the slit pores of mature podocytes, and serves as a basal differentiation marker for podocytes. (Am J Pathol 1995, 147:823–833)

Murine monoclonal antibody (MAb) 5-1-6 causes severe proteinuria when injected into rats.[1] The onset of proteinuria does not involve complement or inflammatory cells and no histological abnormalities are noted in glomeruli except for partial retraction of foot processes at the peak of proteinuria.[1] The target antigen is precipitated from solubilized glomeruli by MAb 5-1-6 and runs as a 51-kd band (p51) on sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions.[1] The epitope identified by MAb 5-1-6 is highly organ and species specific and is detected only in rat glomerular visceral epithelial cells (podocytes, GECs). On immunoelectron microscopy of adult rat kidney sections with MAb 5-1-6, immunoperoxidase reaction product is seen on the surface of foot processes, and immunogold particles appear to be concentrated on the slit diaphragm,[1] which suggests that p51 is a component of the slit diaphragm or its connections to the podocyte plasma membrane. Because severe proteinuria is induced immediately by MAb 5-1-6 binding to p51, and because p51 disappears from the cell surface at the peak of proteinuria,[2] p51 has been considered to be a critical component of the normal filtration barrier.

The slit diaphragm is a continuous bridge-like structure spanning the filtration slit pore between adjacent foot processes of mature GECs. Although its ultrastructural features have been described in detail,[3–5] the molecular composition of the slit diaphragm is still unknown. It appears to originate from a subapical junctional complex connecting immature GECs.[3] As in typical epithelial tight junctions, ZO-1[6]

Supported by National Institute of Diabetes and Digestive and Kidney Diseases grant DK48236 (to DJS), DK34972 (to DRA) and Uehara Memorial Foundation Grant (to HK).

Accepted for publication May 15, 1995.

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is expressed on the cytoplasmic face of this junctional complex and on that of maturing and adult slit diaphragms.[7,8] Slit diaphragms, however, differ from tight junctions both in function in allowing ultrafiltration of large fluid volumes through the slit pores and in extracellular structure.[9] On the other hand, slit diaphragms do have a gate-like function in selectively restricting the passage of plasma macromolecules and in defining the border between the apical domain facing the urinary space and the basolateral domain abutting the glomerular basement membrane and slit pores.[10–12]

To better understand the structural biology of p51 we have studied its biogenesis in the developing glomerulus with a combination of immunolocalization and metabolic labeling techniques. The localization of p51 was compared with that of ZO-1, a component of the cytoplasmic face of the slit diaphragm,[7,8] and with that of podocalyxin, a major component of the glycocalyx and apical marker of the podocyte.[12–14] Our results indicate that p51 is most actively synthesized during glomerulogenesis and is expressed exclusively on the basolateral plasma membrane of developing GECs from the time they first become distinguishable in the S-shaped body.

Materials and Methods

Antibodies

Ascitic fluid containing MAb 5-1-6 was produced in mice primed with 2,6,10,14-tetramethylpentadecane (Sigma Chemical Co., St. Louis, MO) and injected intraperitoneally with a mouse IgG1 hybridoma prepared as previously described.[1] This fluid was subjected to 50% ammonium sulfate precipitation and the immunoglobulin-rich fraction thus obtained was dialyzed against phosphate-buffered saline for 2 days and stored at −80°C. An irrelevant mouse monoclonal IgG1 antibody, RVG1, was used as a control. Rabbit antibody to ZO-1 was purchased from Zymed Laboratories (South San Francisco, CA). Anti-podocalyxin (MAb 5A)[15] was kindly donated by Dr. A. Miettinen (University of Helsinki, Helsinki, Finland).

Animals

Sprague-Dawley and Wistar rats in late pregnancy and normal adult Sprague-Dawley and Wistar rats were purchased from Charles River Laboratories (Wilmington, MA). Newborn rats, 1 to 2 days old, were used.

Immunofluorescence Microscopy

Cryostat kidney sections of approximately 4 μm were fixed with acetone, air dried, and incubated with MAb 5-1-6, followed by incubation with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Cappel-Organon Teknika, Durham, NC). For double-labeling immunofluorescence, sections were stained with anti-ZO-1 antibody and FITC-goat anti-rabbit IgG and followed by staining with MAb 5-1-6 and rhodamine-goat anti-mouse IgG (Cappel-Organon Teknika). Rhodamine-goat anti-mouse IgG was adsorbed with rabbit serum and was confirmed to have no cross-reactivity to rabbit IgG before use. Staining of MAb 5A (anti-podocalyxin MAb) was compared with that of p51 by sequential sections. These sections were examined by epifluorescence microscopy (Optiphot, Nikon, Tokyo, Japan) and photographed with TMAX100 film (Eastman Kodak Co., Rochester, NY).

Immunoelectron Microscopy

For *in vivo* labeling studies, two 2-day-old rats were anesthetized with ether and 0.3 ml of MAb 5-1-6 were injected intravenously via the left saphenous vein. The pups were returned to the litter after recovery from the anesthesia. Twenty-four hours after the first injection, rats were re-anesthetized and then received an intravenous injection of 0.3 ml of goat anti-mouse IgG-horseradish peroxidase (HRP) (1.0 mg/ml IgG (Cappel-Organon Teknika)) through the right saphenous vein. Twenty-four hours after this second injection, rats were again anesthetized and kidneys were fixed *in situ* by the subcapsular injection of 1.6% paraformaldehyde and 3% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4 (Karnovsky's fixative). Tissue slices were then obtained and processed for peroxidase histochemistry and electron microscopy exactly as previously described.[16] Controls consisted of injections of normal mouse IgG-HRP alone.

For post-fixation immunoperoxidase localization studies, kidneys from 2-day-old uninjected rats were fixed with 2% paraformaldehyde, 0.1% glutaraldehyde in 0.1 mol/L phosphate, pH 7.3, for 2 hours and then washed overnight in buffer. Wedges of fixed cortex were equilibrated for 1 hour in 15% sucrose in buffer and frozen on a block of dry ice. The 30- to 40-μm-thick sections were incubated with 40 μg/ml MAb 5-1-6, washed, and then incubated with goat anti-mouse IgG-HRP. Sections were then extensively washed, post-fixed with 2% glutaraldehyde for 15 minutes, and processed for electron microscopy.

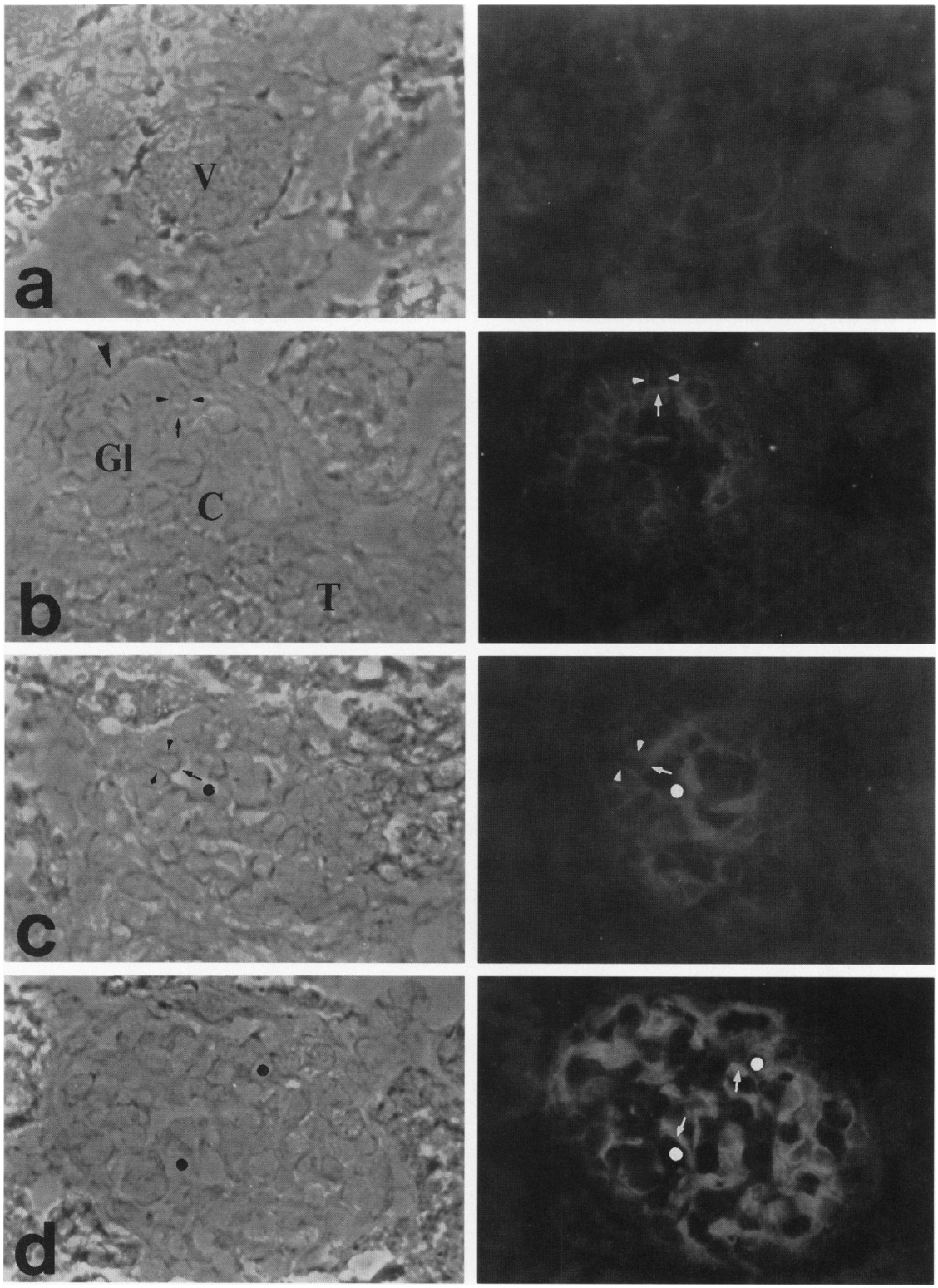

Figure 1. *Phase contrast micrographs of developing glomeruli in kidney sections from a 1-day-old rat and corresponding immunofluorescence micrographs showing staining for p51 with MAb 5-1-6.* **a:** *Renal vesicle. No staining was detected.* **b:** *S-shaped body stage. Faint staining was first detected on the basal (arrow) and lateral (arrowheads) surfaces of the cells destined to become the visceral epithelium.* **c:** *Early capillary loop stage. Staining was observed along the capillary loop (closed circle; arrow) and staining on the lateral surfaces of the epithelial cells (arrowheads) was still detected.* **d:** *Maturing glomerulus. The staining along the basal aspect of epithelial cells lining open capillary loops (closed circles) became progressively broader and more intense with further development (arrows). V, renal vesicle; T, early proximal tubule; Gl, early glomerulus; C, glomerular cleft. Magnification, ×400.*

Metabolic Labeling and Immunoprecipitation

Isolated glomeruli were obtained by sieving renal cortical homogenates through 80- and 140-mesh sieves. Glomeruli from 1-day-old and 5-day-old rats were captured on a 270-mesh (53-μm) sieve and those from adult rats on a 200-mesh (75-μm) sieve. The glomeruli were lightly digested in methionine-free minimal essential medium (MEM) containing 1 mg/ml collagenase (Worthington Biochemical Co., Freehold, NJ) for 30 minutes, dissociated with forceps and passed through a Pasteur pipette (20 times). The tissue was then washed once in methionine-free MEM by centrifugation (1000 × *g* for 1 minute) and preincubated with methionine-free MEM for 30 minutes at 37°C in a 95% air/5% CO_2 incubator with shaking. After one wash, the tissue was labeled with 500 μCi of [^{35}S]methionine in 0.7 ml of methionine-free MEM in a 3.5-cm culture dish. Labeling was carried out in a CO_2 incubator with shaking for 30, 60, 90, or 120 minutes. Labeled tissue was washed in ice-cold Hanks' buffered saline and solubilized in RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate in 50 mmol/L Tris, 150 mM NaCl, pH 8.0) with protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 5 μg/ml soy bean trypsin inhibitor, 4 mmol/L *N*-ethyl

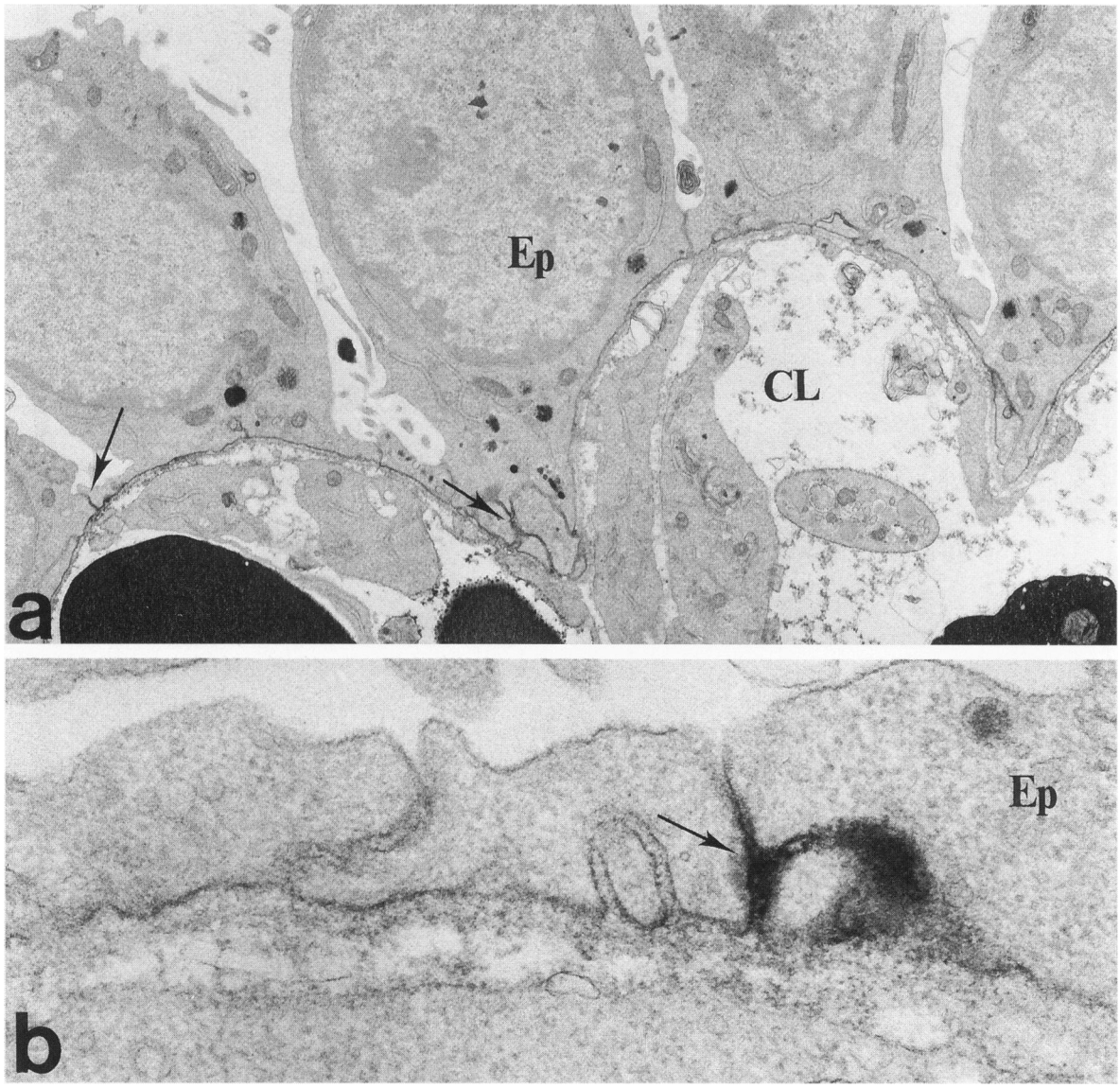

Figure 2. *Immunoperoxidase localization of p51 at the early capillary loop stage in neonatal rat kidneys as determined by sequential injections of MAb 5-1-6 and peroxidase-labeled anti-mouse IgG. Staining for p51 was weakly positive on the basal surface and more strongly positive on the lateral surfaces (arrows) of the immature visceral epithelial cells (Ep) below the level of the occluding junctions. No staining was seen on the apical surface. Note the absence of foot processes. CL, capillary lumen. Magnification, ×13,000 (a); ×68,000 (b).*

maleimide, 5 mmol/L benzamidine hydrochloride). Labeling efficiency was determined from the specific activity (protein-bound counts per microgram of protein). Bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, IL) was used to determine the protein concentration according to the manufacturer's protocol. Samples of equal radioactivity were used for immunoprecipitation to compare the rate of p51 synthesis in 1- and 5-day-old and adult rats. The samples were cleared of insoluble material by centrifugation in a microfuge for 10 minutes at 15,000 × *g* at 4°C. The solubilized sample was precleared with MAb RVG1 (control IgG1), immunoadsorbed with MAb 5-1-6, and precipitated with rabbit anti-mouse IgG and protein A-Affigel (Bio-Rad Laboratories, Melville, NY), and run on sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by autoradiography at −70°C with Kodak X-Omat AR film. After developing, the autoradiographs were scanned on a Molecular Dynamics Personal densitometer (Sunnyvale, CA) and manipulated by using dynamic Image Quart Software (V.3.3) and Adobe Photoshop (V.2.01).

Results

Distribution of p51 in the Developing Glomerulus

When cryostat sections of 1-day-old rat kidney were incubated with MAb 5-1-6, no labeling for p51 was seen on epithelial cells of renal vesicles, which represent the earliest morphological stage of nephrogenesis (Figure 1a). In the more advanced S-shaped bodies, however, p51 was faintly, though clearly, identified on the visceral epithelial cells (early podocytes) adjacent to the vascular cleft. In contrast, parietal (Bowman's capsule) epithelial cells were unlabeled, as were epithelial cells of developing tubules (Figure 1b).

The early capillary loop stage is recognized by the appearance of several capillary loops within the glomerular tuft (Figure 1c). This coincides with the organization of the visceral epithelium into a single layer of cells that are clearly separated by Bowman's space from the squamous parietal epithelium. At this stage, immunofluorescence microscopy showed that p51 was present along the capillary loops but was absent from all other glomerular and extraglomerular structures (Figure 1c). At the ultrastructural level, during the capillary loop stage the visceral epithelial cells are seen to be separated by intercellular spaces that are continuous with Bowman's space. The occluding junctions are located between the visceral epithelial cells near their bases but foot processes are still absent. On *in vivo* labeling immunoelectron microscopy of glomeruli at the capillary loop stage, p51 was seen as weak linear staining of the basal surface of the visceral epithelial cells and was more strongly concentrated on the lateral side below the occluding junctions between the visceral epithelial cells (Figure 2).

With maturation from the late capillary loop stage, the formation of interdigitating foot processes of the visceral epithelium begins. Initially there are only a few interdigitations with broad foot processes, but gradually the interdigitation progresses so that the epithelial foot processes become more numerous and less broad. In the maturing glomeruli, immunofluorescence microscopy showed more intense staining for p51 in a more or less continuous pattern surrounding all capillary loops (Figure 1d). *In vivo* labeling immunoelectron microscopy revealed p51 concentrated in the slit pores below the slit diaphragms and substantially less reaction product of p51 on the basal aspect of the foot processes of maturing glomeruli (Figure 3). No p51 was detected on the apical surface of the foot processes above the slit diaphragms.

Post-fixation indirect immunoperoxidase electron microscopy of normal neonatal rat kidneys gave qualitatively similar findings to those illustrated in Figures 2 and 3, although the intensity of staining was substantially less as a result of the sensitivity of the antigen to a variety of fixatives (data not shown). Nevertheless, we were able to ascertain that the location of immunostaining depicted in Figures 2 and 3 represents the natural distribution of the antigen rather than a redistribution induced by MAb 5-1-6 binding *in vivo*.

Although Wistar rats have more severe proteinuria than Sprague-Dawley rats when injected with MAb 5-1-6 (see Note Added in Proof), we found no differences of p51 staining at any stage between Wistar and Sprague-Dawley rats.

Localization of p51 Is Different from That of ZO-1 or Podocalyxin

To further define the localization of p51, we compared its distribution to that of podocalyxin, a marker of the GEC apical plasma membrane, and ZO-1, a component of the cytoplasmic face of mature slit diaphragms and of primitive GEC junctional complexes. In sequential sections of the same developing glomeruli, podocalyxin and p51 were seen on

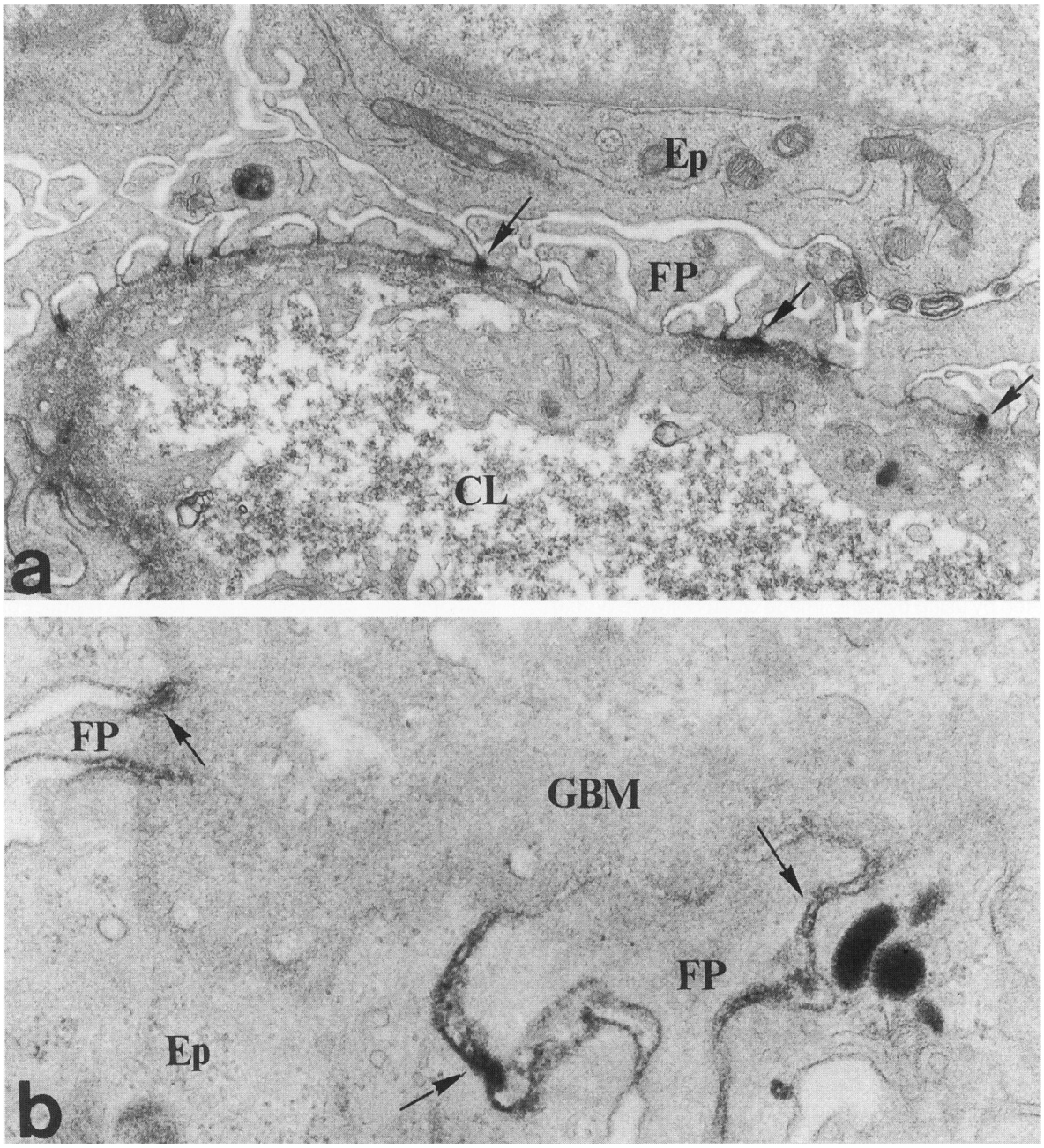

Figure 3. *Immunoperoxidase localization of p51 in the maturing glomerulus in neonatal rat kidneys as determined by sequential injections of MAb 5-1-6 and peroxidase-labeled anti-mouse IgG.* a *and* b: *With the formation of interdigitating foot processes (FP), p51 became restricted in the slit pores (arrows).* b: *Oblique section across the glomerular capillary wall. Note localization of p51 mainly to the slit pores and not to the soles of foot processes. Ep, visceral epithelial cells; CL, capillary lumen; GBM, glomerular basement membrane. Magnification, ×30,000* (a); *×48,000* (b).

opposite surfaces of the epithelial cells. At the S-shaped body stage, when a fluorescent signal for p51 was first observed on the basal and lateral sides of the developing glomerular epithelium (Figure 4a), podocalyxin was found along the apical and lateral sides (Figure 4b). The same distribution of podocalyxin to that shown in Figure 4b was also seen at the capillary loop stage and during further maturation.

Although dual-labeling studies demonstrate that p51 and ZO-1 are in close proximity in maturing glomeruli (Figure 5), their patterns of distribution are different and they appear to arrive at their final locations from different origins. As described by Schnabel et al,[7] ZO-1 was first detected on the cells destined to become visceral epithelial cells at the S-shaped body stage as faint fluorescent dots. Dot-

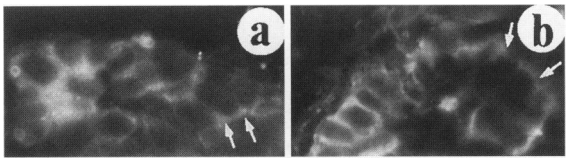

Figure 4. *Localization of p51 and podocalyxin in a developing glomerulus at the S-shaped body stage. Sequential sections were stained with MAb 5-1-6* (a) *and anti-podocalyxin* (b). *In regions where the glomeruli were cut in cross section, p51 and podocalyxin were detected on opposite surfaces of the epithelial cells. In* a, *the arrows indicate basolateral staining of p51, and in* b, *they point to apical and lateral staining for podocalyxin. Magnification, ×400.*

and fleck-like staining for ZO-1 was also seen at all stages at the junctions between parietal and tubular epithelial cells. In contrast, p51 was never observed on any cells other than developing podocytes. From the capillary loop stage to the maturing stage, ZO-1 labeling became visible as a series of dots or an interrupted line surrounding capillary loops (Figure 5a). At the same focal plane, p51 was often, but not always, seen as a continuous band adjacent to ZO-1 (Figure 5b). Slight adjustments to the plane of focus suggested that ZO-1 has a more restricted distribution than p51 and that p51 lies slightly below ZO-1.

Biosynthetic Labeling of p51 in Developing and Mature Glomeruli

To examine the synthesis of p51 during glomerular development, we took advantage of the fact that neonatal rat kidneys contain a high proportion of developing glomeruli. Incorporation of [^{35}S]methionine into immunoprecipitable p51 by isolated neonatal (1-day-old) rat glomeruli was compared with that by glomeruli from 5-day-old and adult rats. The labeling efficiency of 1- and 5-day-old and adult rat glomeruli after a 120-minute pulse of 500 μCi of [^{35}S]methionine was 19,972, 4,469, and 2,475 cpm/μg protein, respectively. A strong 55-kd band and two weak bands of 43 and 46 kd were consistently precipitated by MAb 5-1-6 from the metabolically labeled samples of 1-day-old rat glomeruli (Figure 6). No bands were detected in the labeled samples from 5-day-old or adult rat glomeruli, despite loading equivalent amounts of protein-bound radioactivity. Identical immunoprecipitation results were obtained with Wistar and Sprague-Dawley rat glomeruli.

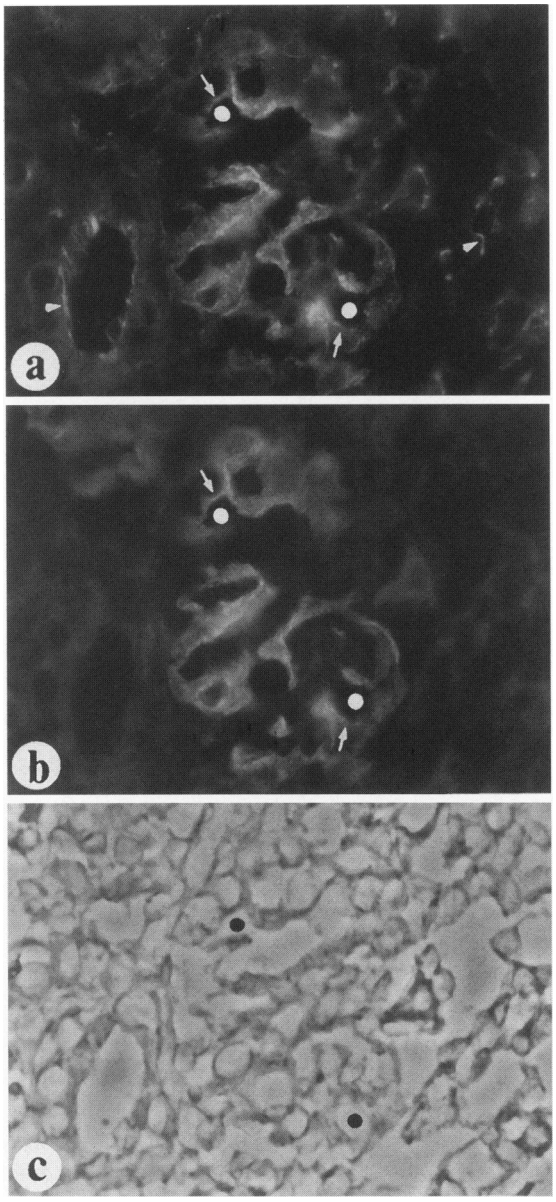

Figure 5. *Localization of ZO-1 and p51 in the maturing glomerulus. Dual immunofluorescent labeling was carried out with polyclonal anti-ZO-1 and MAb 5-1-6, followed by FITC- and rhodamine-labeled secondary antibodies, respectively. Anti-ZO-1 was used to identify the location of the slit diaphragms. In these micrographs the same glomerulus was photographed focusing on ZO-1* (a) *and then p51* (b) *was photographed without changing focus. ZO-1 was observed as a discontinuous row of discrete fluorescent dots in the visceral epithelial cytoplasm* (a, *arrows*). *At this level, p51 was visible as a discontinuous band on the visceral epithelial cell surface* (b, *arrows*), *but it was more clearly seen at a slightly lower plane of focus. ZO-1 was also detected at the junctions between parietal and tubular epithelial cells* (a, *arrowheads*). *By contrast, p51 was detected only on visceral epithelial cells.* c: *Phase contrast microscopy at the same plane. Closed circle, capillary loop; magnification, ×400.*

Discussion

Although GECs and their intervening slit diaphragms appear to play an important role in maintaining normal glomerular permeability and hydraulic conductivity,[9,17] little is known about their structural biology and molecular composition. On the premise that p51 is an essential component of the filtration barrier, we have begun a detailed analysis of this protein as a

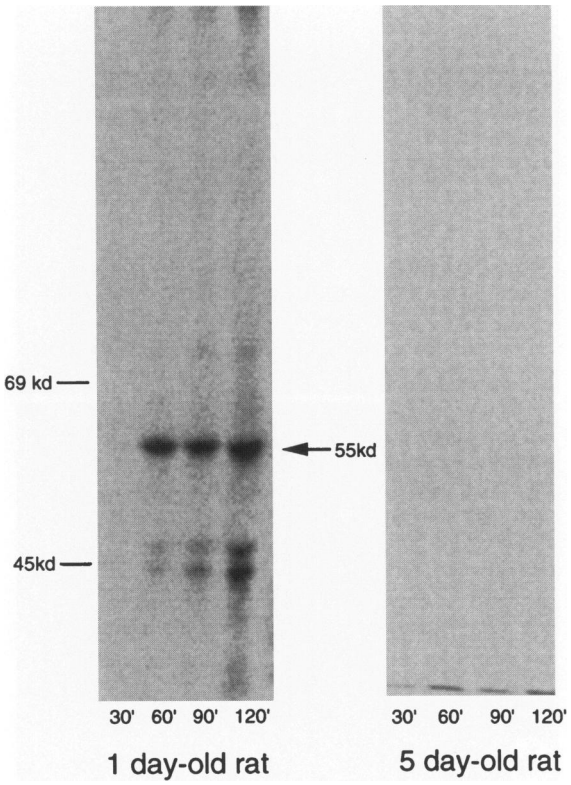

Figure 6. *Immunoprecipitation of glomerular proteins with MAb 5-1-6. Isolated glomeruli from 1-day-old and 5-day-old rats were pulse-labeled with [^{35}S]methionine for 30, 60, 90, or 120 minutes and immunoprecipitated with MAb 5-1-6. A clear band of 55 kd and two weaker bands of 43 and 46 kd were detected in 1-day-old rat glomeruli. No bands were detected in 5-day-old rat glomeruli.*

means of gaining insight into the normal structure and function of the podocyte.[1,2,18–20] Our working hypothesis is that p51 is an integral transmembrane protein of the slit diaphragm or the adjacent podocyte plasma membrane, and the binding of antibody to this component alters the molecular arrangement that constitutes a normal functioning slit diaphragm. In this study we examined the biogenesis of p51 in the developing glomerulus by immunohistology and immunoprecipitation and obtained some intriguing and novel findings.

First, we found that there is a close relationship between the concentration of p51 and the maturation of foot processes. Based on our observations on p51, and taking into account what is already known about glomerular development,[5,21,22] the maturation of GEC foot processes and slit diaphragms is understood as follows. Interdigitation of adjacent epithelial cells begins at the late capillary loop stage. Initially there are only a few interdigitations with broad processes but gradually the interdigitation progresses so that the epithelial processes become more numerous and less broad. p51 is observed along the basal and lateral sides of the podocytes at the early capillary loop stage before the onset of interdigitation. With further maturation of the podocyte, p51 becomes concentrated in the slit pore. It is presently unknown whether p51 participates in the formation of the foot processes. It is interesting to note, however, that immunostaining for p51 is altered during the proteinuric phase of glomerular epithelial injury induced by either aminonucleoside of puromycin or adriamycin at a time when diffuse effacement of foot processes is seen.[23] This suggests that p51 may be essential for maintaining the normal architecture of the foot processes.

Whereas immunofluorescence microscopy suggests a more or less continuous distribution of p51 along the podocyte basolateral surface of immature and maturing glomeruli, our immunoperoxidase findings indicate a more restricted localization in the filtration slit pores on or below the slit diaphragms in the more mature glomeruli. We believe this difference probably reflects the greater resolving power of immunoelectron microscopy of ultrathin sections and the superpositioning of labeling in 4-μm-thick cryostat sections. It is important to consider the alternative possibility, however; that is, that the antigen is normally more uniformly expressed along the bases of the foot processes abutting the basement membrane and undergoes redistribution into the slit pores after antibody cross-linking *in vivo*, as suggested by immunofluorescence.[1] This explanation seems less likely because redistribution takes longer to occur than the 2 days between antibody injection and tissue fixation in these studies,[1] and because indirect immunoperoxidase studies of normal kidneys *in vitro* gave qualitatively similar results to the *in vivo* labeling approaches. Thus we maintain that the concentration of p51 in the slit pores corresponds to maturation of the foot processes and formation of the slit diaphragms.

Despite the close proximity of p51 and ZO-1 in the maturing GEC, our findings indicate that they originate from different parts of the cell. Published immunoelectron microscopy data indicate that ZO-1 decorates the cytoplasmic face of mature slit diaphragms[7] and that p51 is located on the slit diaphragm itself.[1] On the other hand, ZO-1 and p51 appear to arrive at their final locations from opposite directions. Based on the immunolocalization of ZO-1, Schnabel and her co-workers have regarded the slit diaphragm as a variant of a tight junction.[7] ZO-1 is detected along the cytoplasmic surface of occluding junctions of all epithelial cells and consists of two isoforms that differ in the presence of motif-α (ZO-1α^+, ZO-1α^-).[8] Only one isoform, lacking motif-α

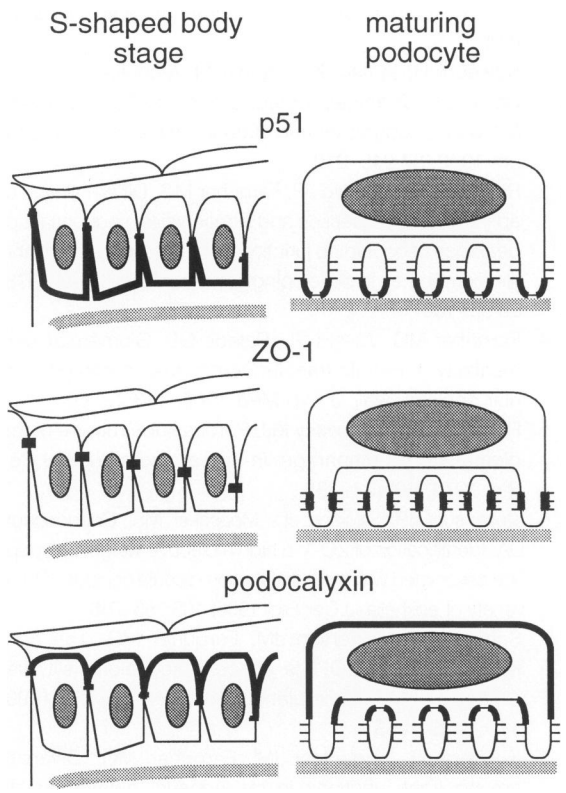

Figure 7. *Schematic illustration of the glomerular epithelial cell distribution of p51, ZO-1, and podocalyxin during glomerular development. During maturation from the S-shaped body stage onwards, the junctional complexes migrate along the lateral cell membranes toward the basal surface. In the mature podocyte, the slit diaphragms are formed between foot processes. At the S-shaped body stage, p51 is detected on the basal and the lateral sides below the junction. In the mature podocyte, p51 becomes concentrated in the slit pores on or below the slit diaphragm, whereas staining at the bases of the foot processes becomes weak or negative. ZO-1 is detected in the cytoplasm opposite junctional complexes at the S-shaped body stage and opposite the slit diaphragms in mature podocytes.[7] Podocalyxin is detected on the apical plasma membrane above the occluding junction at the S-shaped body stage and above the slit diaphragm in the mature podocyte.[12]*

(ZO-1α^-), is expressed in the highly specialized slit diaphragms.[8] During development, the subapical junctional complexes between primordial podocytes migrate along the lateral cell margins toward the basal surface and disappear coincident with the opening of the intercellular spaces and the appearance of slit diaphragms. ZO-1α^- remains present throughout these processes. In contrast, p51 is present along the basal surface of the immature podocyte and becomes concentrated in the slit pore at or below the slit diaphragm (illustrated in Figure 7). In further contrast to ZO-1, p51 is highly specific for podocytes. Thus, p51 appears to have little developmental relationship with the embryonal GEC tight junction. Although there is no definitive explanation for this apparent discrepancy, there are at least two possibilities. First, because immunogold techniques require bridging antibodies and provide only approximate localization of the target antigen, it is possible that either p51 or ZO-1 lie close to, but are not actual constituents of, the slit diaphragm. Second, it is conceivable that the slit diaphragm is assembled *in situ* from components of both the apical and basolateral plasma membranes. The final answer awaits full characterization of the components of the slit diaphragm.

By comparing the localization of p51 and podocalyxin during glomerulogenesis we were able to gain additional information on the distinctive origin of p51. Podocalyxin, the major anionic glycoprotein of the GEC surface,[13] is confined to the apical and lateral surfaces above the embryonal occluding junctions[12] and mature slit diaphragms.[14] Adjacent sections of developing and mature kidneys displayed staining for p51 and podocalyxin on opposite sides of the glomerular epithelium from the S-shaped body stage onwards. At no stage was p51 seen on the side facing Bowman's space. Several other rat podocyte antigens have been isolated[15] and characterized, including gp330,[24–27] podoendin,[28] DPP IV,[29,30] 115/107-kd protein,[31] pp44,[32] *O*-acetylated GD3,[33] and 13A antigen.[34] Among all of these antigens, however, p51 is the first to be identified as a unique podocyte basal marker.

Metabolic labeling studies showed that p51 is actively synthesized in the early phase of glomerular development and that the rate of synthesis declines with maturation. A clear 55-kd band and weak bands of 43 and 46 kd were consistently detected in glomerular isolates from 1-day-old rats, which contain a high proportion of immature glomeruli (Figure 6). We believe that the 55-kd band corresponds to the 51-kd protein originally identified by surface labeling of solubilized adult rat glomeruli.[1] The differences in apparent size may be purely technical but it is possible that metabolic labeling identifies a relatively abundant intracellular pool of unprocessed precursor protein. However, the fact that we did not observe the appearance of a second, smaller (51-kd) protein band despite incubation for up to 24 hours (data not shown) is somewhat against this possibility. Until definitive information on the primary structure of the protein is available, we have chosen to retain the name p51 based on the original report.[1] The nature of the accompanying 43- and 46-kd bands is unknown. They may be degradation products of the 55-kd protein or co-precipitating, noncovalently associated proteins.

Existing data from these and previous studies lead us to the conclusion that p51 in normal mature GECs is a stable membrane-associated protein with low

metabolic turnover. GEC membrane staining for p51 at the S-shaped body stage is faint, but staining increases progressively with maturation whereas the synthetic rate declines. The total protein labeling efficiency of 5-day-old glomeruli was about one-fifth of the 1-day-old rat samples; that of adult rat glomeruli was one-eighth. To compare p51 synthetic rates at the different stages, materials of the same radioactivity were used for immunoprecipitation. Thus the absence of bands in the 5-day-old and adult rat immunoprecipitates is a true reflection of a low level of p51 synthesis rather than a deficiency of labeled proteins. This information will likely provide a useful basis for understanding the turnover of p51 after MAb 5-1-6 binding *in vivo*. Thus, when cross-linked by divalent MAb 5-1-6, p51 redistributes and disappears from the cell surface.[2,20] Concomitantly, the antibody appears to be internalized into multivesicular bodies after binding its target antigen.[1,18] The calmodulin-cytoskeleton inhibitor, chlorpromazine, has no effect on either immune deposit redistribution or proteinuria.[19] With recovery, the antigen reappears on the cell surface as proteinuria subsides.[18] In future studies we hope to determine whether this recovery is a result of *de novo* synthesis or recycling of endocytosed p51.

We conclude that p51, the target of the nephritogenic antibody MAb 5-1-6, is primarily synthesized during glomerular development. It first appears on the basal surface of GECs at the earliest stage at which this cell type can be recognized morphologically and becomes concentrated in the slit pores of mature podocytes. As such, p51 serves as a basal differentiation marker for podocytes.

Note Added in Proof

Since submission of this manuscript, a study documenting the variability of different rat strains to the effects of MAb 5-1-6 has been accepted for publication.[35]

Acknowledgments

Portions of this work were presented in part at 27th Annual Meeting of the American Society of Nephrology in Orlando, FL, and published in abstract form (J Am Soc Nephrol 5:753, 1994).

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