Spatiotemporal expression of molecules associated with junctional complexes during the in vivo maturation of renal podocytes

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ABSTRACT Epithelial glomerular cells differentiate from mesenchymal cells of the metanephrogenic blastema. During the first stages of glomerulogenesis, the cells acquire the morphological features of epithelial cells. Then, podocytes lose these characteristics at the maturing glomerular stage. We have studied the molecules associated with junctional complexes during glomerular differentiation in human and pig fetal kidneys. We show for the first time the expression of P-cadherin in renal cells. Epithelial cells of ureteral buds and ampullae display all the molecules associated with junctional complexes and coexpress E- and P-cadherin. However, P-cadherin, plakoglobin and vinculin are the only markers detected in future glomerular cells. We have established a spatiotemporal correlation between the time of appearance and disappearance of junctional complexes as previously described (Saxén and Wartiovaara, Int. J. Cancer 1:271-290, 1966; Saxén et al., Adv. Morphog. 7:251-293, 1968; Reeves et al., Lab. Invest. 39:90-100, 1978), and the expression of their associated molecules. Epithelial cells with stable, typical junctional complexes strongly express the molecules associated with junctions, whereas cells endowed with transient, atypical junctional complexes express low amounts of components associated with junctions. These observations suggest a correlation between the level of expression of these components and an authentic, stable epithelial phenotype.

KEY WORDS: cell adhesion molecules, junctional complexes, cytoskeleton, differentiation, podocytes

Introduction

Podocytes are the visceral epithelial cells of the glomerulus. They differentiate from mesenchymal cells of the metanephrogenic blastema by sequential steps, depending on ureteral bud induction (Grobstein, 1956). The first sign of epithelial differentiation is the clustering in caps of induced mesenchymal cells. The formation of vesicles and subsequent developmental stages (S-shape body, capillary loop and maturing glomeruli) have been described previously (Zamboni and De Martino, 1968; Reeves et al., 1978; Holthöfer et al., 1984). At the renal vesicle stage, the precursors of glomerular and tubular cells cannot be identified. Conversely, at the S-shape body stage, the glomerular and tubular parts of the future nephron may be distinguished, and the two types of glomerular epithelial cells are clearly separated.

The process of nephron differentiation results in changes in cell phenotype, interrelationship and adhesive properties. The mesenchymal cells of the metanephric blastema are irregular in shape, establish no close intercellular contacts, exhibit no definite orientation (Saxén and Wartiovaara, 1965) and adhere to extracellular matrix via substrate adhesion molecules (SAMs). In contrast, membranes of epithelial cells are in close contact and are connected by junctional complexes which may be tight or occluding junctions, zonula adherens, or macula adherens, as initially defined on morphologic and/or functional grounds (Farquhar and Palade, 1963; Staehelin, 1974). Junctional complexes comprise three different domains: the membrane, the submembrane cytoplasm and the cytoskeleton, which are now characterized by the presence of molecules specific for each type of junctions (Volk and Geiger, 1984). The transmembrane components of the tight junctions are not yet known. The Ca++ dependent cell adhesion molecules (CAMs or cadherins) are cell surface glycoproteins in adherens junctions which function as cell contact receptors (Boller et al., 1985; Volk and Geiger, 1986a,b; Takeichi, 1987). The role of A-CAM (adherens-junction-specific cell adhesion molecule) in the

Abbreviations used in this paper: SAMs, substrate adhesion molecules; CAMs, cell adhesion molecules; FITC, fluorescein isothiocyanate; PBS, phosphate buffered saline.
Fig. 1. Indirect immunofluorescence of metanephrogenic blastemal mesenchymal cells and inductive elements. (A) The anti-vinculin antibody reveals numerous very bright dots. Note the shallow staining seen as bright dots in the apical zone of the lateral membranes of the ampullar cells (a) and the strong immunoreactivity in basal membranes. (B) The mesenchymal cells are strongly vimentin positive. Epithelial cells of inductive elements: ampullae (a) and ureteral buds (ub) also express intermediate filaments of vimentin type. (C) Cytokeratins are not expressed in mesenchymal cells (mc), whereas ureteral buds (ub) and ampullar cells (a) display a strong expression of these proteins. (D) Numerous actin microfilaments are detected in mesenchymal cells with phallolidin, which also reveals the presence of actin in lateral cell borders of inductive elements (a, ub). Note the accumulation of actin in the apical cell parts, and its pavement-like aspect in cross sections. Bar, 8 μm. (E) In ureteral buds (ub) and ampullar cells (a), the ZO-1 protein appears as tiny bright dots in the apical region of the lateral cell membranes, near the lumen. In cross sections, it is visualized as a ring at the cell periphery. (F) The ureteral buds (ub) and ampullar cells (a) are decorated with antibody to E-cadherin. The reactivity decreases from the apicolateral to the basolateral part of the membranes. It is absent from the apical cell membrane near the lumen. (G) A strong expression in a similar pattern of localization is seen in ureteral buds (ub) and ampullar cells (a) with antibody directed against P-cadherin. (H) The cell borders of ampullae (a) and ureteral buds (ub) are positively labeled with antiplakoglobin antibody. It displays a pavement-like aspect, interrupted on the superficial cell membrane near the lumen. See also Fig. 2C for the immunoreactivity of the ampullar cells (a) with this antibody. (I) Desmoplakin staining appears as tiny, bright dots at the apical part of lateral membranes of the ampullar cells (a). Bar, 12 μm.
The process of cell aggregation has been established by Duband et al. (1988) who demonstrated a spatial and temporal coordination between the expression of A-CAM and epithelialization, or between its disappearance and the process of de-epithelialization. Desmocollins and desmogleins are transmembrane cadherin-like molecules found in desmosomes (Mechanic et al., 1991; Arnemann et al., 1992). The ZO-1 protein belongs to the cytoplasmic domain of tight junctions (Schnabel et al., 1990), whereas plakoglobin (Cowin et al., 1986) and vinculin (Geiger et al., 1983, 1985) are cytoplasmic components of adherens junctions. Vinculin is also involved in matrix cell interactions (Geiger et al., 1985). Plakoglobin is also present in the desmosomal cytoplasmic plaque (Cowin et al., 1986), in association with desmoplakin, which is specific for this domain (Mueller and Franke, 1983). Finally, tight junctions are connected to actin filaments (Meza et al., 1980; Madara 1987), presumably via ZO-1 protein (Anderson et al., 1988). E-cadherin/uvomorulin is coupled to actin via a vinculin linkage (Geiger, 1979; Geiger et al., 1981, 1983; Isenberg et al., 1982; Wilkins and Lin, 1982; Volk and Geiger, 1986a; Hirano et al., 1987). The desmosomal plaque is anchored to intermediate filaments. Intermediate filament proteins are cell-type specific and cytokeratins are typical of epithelial and mesothelial cells (Franke et al., 1978, 1982a; Moll et al., 1982; Quinlan et al., 1985; Cardo et al., 1987; Kasper et al., 1987). They are present in almost all epithelial cells both in vivo and in vitro (Sun et al., 1983; Markl, 1991), whereas vimentin is expressed by mesenchymal cells (Franke et al., 1982a; Moll et al., 1982; Scheffer et al., 1982; Quinlan et al., 1985; Kasper, 1989).

Fig. 2. Vesicle (v) cells observed by immunofluorescence microscopy. On each section the inducing ampulla is located beside the vesicle. (A) E-cadherin is present in the upper part of the lateral cell membranes. (B) Note the strong expression of P-cadherin, compared to that of E-cadherin, with a bright fluorescence near the lumen. Bar, 8 µm. (C) Plakoglobin is present in low amounts. (D) These cells display faint staining with anti-vinculin antibody, which is restricted to the apical part of the lateral cell membranes. Bar, 12 µm. (E) The cytoplasm of all vesicle cells is stained with anti-vimentin antibody. Bar, 6 µm. (F) In elongated vesicles, the reaction with anti-vimentin antibody shows a gradient with cells less stained in the upper part than in the lower zone of the vesicle. (G) Certain vesicles are completely devoid of cytokeratins, whereas in H beginning of expression is detected with the antibody to cytokeratins. Bar, 8 µm. (I) Actin filaments are mainly seen near the lumen, and sometimes near lateral cell membranes. Bar, 12 µm.
Fig. 3. Immunofluorescence microscopy of S-shape bodies. At the S-shape body stage, glomerular epithelial cells are generally faintly immunoreactive with all antibodies. (A, B, C, G) Cross sections of S-shape bodies. (A) The two epithelial cell types in the lower limb (II) of the S-shape body express P-cadherin in the apical region of their lateral membranes. Bar, 8 μm. (B) The same cells show plakoglobin immunoreactivity. Endothelial cells (arrows) are also labeled with the antibody. (C) The apical parts of both future epithelial glomerular cells in the lower limb (II) of the S-shape body are decorated with anti-vinculin antibody. A punctiform fluorescence is also seen in their basal membranes. (D) Note the gradient of vimentin expression from the distal extremity (de) towards the proximal tubule (pt). (E) An inverse gradient in cytokeratin expression is observed at the beginning of this stage. (F) Later this gradient distribution disappears. Note the stronger expression of keratin in presumptive podocytes (pp) than in future parietal (fp) epithelial cells. (G) Phalloidin is present as a peripheral ring, the most intense staining is at the upper and lower parts of the cells. Bar, 12 μm.

Junctions are present between developing podocytes, and disappear with maturation (Reeves et al., 1978). E-cadherin/uvomorulin has not been detected in these cells (Vestweber et al., 1985). The transient expression of cytokeratins has been reported (Moll et al., 1991), but was not seen by Holthöfer et al. (1984).

In the present study high affinity, specific antibodies have been used to establish the spatiotemporal pattern of expression of junction-associated proteins during podocyte differentiation in human and pig embryos. The presence or absence of these molecules has been correlated with the appearance and disappearance of junctions that have been previously described (Saxén and Wartiovaara, 1966; Saxén et al., 1968; Reeves et al., 1978).

Results

The same pattern of immunolabeling was observed in human and pig fetal kidneys, with all antibodies.

Melanephrogenic blastema

Uninduced and condensed blastemal mesenchymal cells displayed no immunoreactivity with the antibodies to ZO-1 protein, E- and P-cadherin, and plakoglobin. Mesenchymal cells were also desmoplakin negative and remained negative during their differentiation into epithelial cells. Numerous very bright immunofluorescent dots were seen in blastemal mesenchymal cells incubated with antibody to vinculin (Fig. 1A). These cells also displayed a bright vimentin immunoreactivity, which was even stronger in cells clustered in caps around the ampullae (Fig. 1B). In contrast, all mesenchymal cells, both uninduced or clustered in caps, were keratin negative (Fig. 1C). Phalloidin labeling revealed a network of short filaments in the melanephrogenic blastemal cells (Fig. 1D).

The basolateral membrane of endothelial cells lining capillaries and arteries was brightly stained by plakoglobin antibodies throughout kidney differentiation.

Ureteral buds and ampullar cells

Ureteral buds and ampullar cells were positively labeled with the antibody to ZO-1 protein. The staining was seen as tiny and very bright dots in the apical part of lateral cell membranes, and as a continuous line around cells in cross sections (Fig. 1E). The lateral cell membranes were strongly decorated with antibodies directed against E- and P-cadherin. The two markers were co-localized in
**Fig. 4.** Capillary loop and maturing glomerular stages. (A) P-cadherin is present at the basal pole of podocytes (p). It is heterogeneously distributed in the Bowman's capsule cells (Bc). (B) Plakoglobin is seen as a very bright line at the level of the glomerular basement membrane with spiny extensions towards the podocytes (p). Some parietal epithelial cells are also labeled with this antibody. (C) The basal parts of podocytes (p) display strong vinculin reactivity. There is intense punctiform labeling on the Bowman's capsule cells (Bc). Bar, 12 μm. (D) Strong expression of vimentin is seen as bright patches on the basal part of podocytes, whereas a filamentous arrangement of vimentin outlines the periphery of podocytes (p). Parietal epithelial cells (pc) are also brightly decorated with anti-vimentin antibody. (E) Keratin expression appears as residual patches in some basal areas of podocytes (p). It is not regularly distributed in Bowman's capsule cells (Bc), although its expression increases in these cells. Bar, 8 μm. (F) Both epithelial cell types are stained with phalloidin. A stronger fluorescent signal in bright patches is observed at the level of attachment of podocytes to the glomerular basement membrane, whereas the periphery of podocytes displays only discrete staining. Bar, 12 μm.

a polygonal-shaped pattern in cross sections (Fig. 1F,G). They were absent from the apical membranes (Fig. 1F,G). Ureteral bud cells expressed plakoglobin immunoreactivity in a typical honeycomb-like pattern delineating the lateral membranes (Fig. 1H). Labeling was restricted to the apical zone of lateral membranes in ampullar cells (Fig. 2C). The distribution of desmoplakin was similar, but in contrast to plakoglobin labeling, it was seen as bright punctate dots at the periphery of the cells (Fig. 1I). The lateral membranes of ureteral buds and ampullar cells were strongly vinculin positive, especially at their apical part (Fig. 1A). Condensation of vinculin was also observed along their basal membrane (Fig. 1A). These epithelial cells expressed vimentin and keratin, both appearing as cytoplasmic filaments running through the cells, with condensation of keratin filaments along the plasma membrane (Fig. 1B,C). In ureteral buds and ampullar cells, actin labeling was cortical (Fig. 1D).

**Vesicle stage**

The ZO-1 protein was not detected in vesicle cells or at any stage of glomerulogenesis. Vesicle cells displayed apical labeling with antibodies to E-cadherin (Fig. 2A) and P-cadherin (Fig. 2B). A weak fluorescence was detected near the apical pole of vesicle cells stained with anti-plakoglobin antibody (Fig. 2C). The intensity of vinculin staining was considerably lower in vesicle cells than in ureteral buds and ampullar cells (Fig. 2D) with immunoreactivity only in the apical part of the lateral cell membranes (Fig. 2D). There were intracellular filaments of the vimentin type in the majority of vesicles, especially in the lateral and basal domains of cells (Fig. 2E). At the end of this stage, the first differences were observed between the upper and lower regions of the vesicle. Cells of the upper zone, near the ampullae became less vimentin positive, whereas cells located in the lower zone remained strongly positive (Fig. 2F). At this stage, the staining of cytokeratins was variable. Some vesicles were entirely negative (Fig. 2G), while others displayed faint, randomly scattered immunoreactivity (Fig. 2H). Keratin expression therefore appeared to be superimposed to that of vimentin in vesicle cells. The cytoplasmic actin filaments in these cells were clustered in bundles near the apical and lateral cell membranes (Fig. 2I).

**S-shape body stage**

E-cadherin/uvomorulin was not detected in the two cell types of the lower limb of the S-shape body, whereas, at the same stage, the presumptive parietal and visceral glomerular cells were decorated,
in a ring fashion, with antibody to P-cadherin (Fig. 3A). These cells exhibited peripheral plakoglobin immunoreactivity (Fig. 3B). Vinculin was present in the apical and basal parts of both epithelial cell types (Fig. 3C) of the lower limb of the S-shape body.

Vimentin was still present in the lower limb of the S-shape body but the expression was very low and restricted to the more distal cells (Fig. 3D). Cells located in the middle and proximal parts of the lower limb of the S-shape body expressed keratin (Fig. 3E). The differences in keratin expression between the different parts of the lower limb disappeared with maturation; all cells became brightly keratin positive (Fig. 3F). The two cell types of the lower limb of the S-shape body were also actin positive; the staining was concentrated in the apical and basal cellular domains (Fig. 3G).

Developing capillary loop and maturing glomerulus stages

During the capillary loop and maturing glomerulus stages, E-cadherin/uvomorulin was seen in parietal epithelial cells of Bowman’s capsule, whereas intense P-cadherin immunolabeling was observed in parietal glomerular epithelial cells and podocytes (Fig. 4A). Immunolabeling was more intense in the foot process region of the podocytes, near the glomerular basement membrane (GBM), than in the lateral parts of the cells; it was discontinuous in parietal glomerular epithelial cells (Fig. 4A). Intense plakoglobin immunoreactivity was seen as a very bright line along the external aspect of the GBM, with spiky extensions directed towards the podocytes. The parietal epithelial cells of Bowman’s capsule were also decorated with this antibody (Fig. 4B). At the capillary loop stage, a faint residual vinculin immunolabeling was observed at the basal part of podocytes along the GBM, and a short straight fluorescent line perpendicular to the GBM could be focally seen, presumably between podocytes not yet completely separated. The striking loss of vinculin immunostaining in podocytes was even more evident in maturing glomeruli. In contrast, parietal glomerular epithelial cells remained strongly vinculin positive (Fig. 4C). Vimentin immunostaining was patchy and restricted to the basal domain of podocytes. Vimentin filaments were progressively oriented parallel to the long cell axis, and concentrated along the lateral cell border. Parietal glomerular epithelial cells were still strongly immunoreactive (Fig. 4D). At the maturing glomerulus stage, keratin immunostaining of podocytes was dramatically reduced. Some podocytes were even completely devoid of immunoreactivity. Parietal epithelial cells of Bowman’s capsule contained strong, but heterogeneous

Fig. 5. Mature glomeruli. (A) Note the presence of E-cadherin only in some parietal epithelial cells (pc). (B) A weak staining with anti P-cadherin antibody is present in parietal (p) and visceral (v) epithelial cells. (C) Plakoglobin immunoreactivity is seen as short strings along the glomerular basement membrane, and in the Bowman’s capsule cells (Bc). (D) Vinculin is no longer expressed by podocytes whereas it is seen as tiny, bright dots in parietal epithelial cells (pe). Bar, 12 μm. (E) High amounts of vimentin are expressed in both epithelial cell types of mature glomeruli. Bar: 6 μm. (F) Note the complete loss of keratin expression in mature podocytes (p), and its strong, although heterogeneous distribution in parietal epithelial cells (pc). (G) Actin is present in Bowman’s capsule cells (Bc), whereas mature podocytes no longer express it. Bar, 12 μm. Vinculin, vimentin and actin are strongly expressed by glomerular endocapillary cells.
staining (Fig. 4E). The basal and apical actin localization previously observed was maintained in podocytes during the developing capillary loop and maturing glomerulus stages (Fig. 4F).

**Mature glomeruli**

The E-cadherin immunostaining persisted in parietal epithelial cells of Bowman’s capsule of mature glomeruli (Fig. 5A). The intensity of P-cadherin immunolabeling was dramatically reduced in the mature glomeruli compared to the earlier stages: it was found along the basal but not the lateral membranes of podocytes, and was occasionally seen in parietal epithelial cells (Fig. 5B). The spurious aspect of the plakoglobin labeling observed in the preceding stages was entirely lost in differentiated podocytes. Plakoglobin was retained along the capillary endothelial cells as well as in some parietal glomerular epithelial cells (Fig. 5C). Vinculin immunostaining was seen in parietal epithelial cells of Bowman’s capsule, but not in mature podocytes (Fig. 5D). Vimentin-positive immunostaining persisted and even increased in podocytes at the mature glomerular stage. Parietal epithelial cells of Bowman’s capsule were faintly but clearly positive for vimentin (Fig. 5E). Since mesangial and endothelial cells also express vimentin, the entire mature glomeruli were strongly fluorescent (Fig. 5E). Mature podocytes were keratin-negative (Fig. 5F). The parietal epithelial cells of some Bowman’s capsules were entirely keratin-positive, whereas other capsules were heterogeneously labeled (Fig. 5F). Mature glomeruli had phalloidin-positive capsular cells, whereas podocytes had lost their reactivity (Fig. 5G).

**Discussion**

In the present study, we analyzed the spatiotemporal distribution of molecules associated with junctional complexes in the developing glomeruluses of human and pig fetuses, compared with the ureteral buds and ampullae. The same pattern of expression was observed in the two species.

In epithelial cells of ureteral buds and ampullae we observed a strong expression of all junction-associated molecules of epithelial tissues, ZO-1 protein, E-cadherin/uvomorulin (Vestweber et al., 1985; Saxén et al., 1988), plakoglobin, desmoplakin, vinculin. We show here for the first time that P-cadherin previously observed in other epithelial structures (Takeichi, 1987) is also present in the ureteral buds and ampullary cells.

Mesenchymal cells of the metanephric blastema are vimentin-positive, express vimentin but not cytokeratins, and possess numerous actin microfilaments. These cells, whether uninduced or clustered in caps, are negative with all antibodies detecting the molecules associated with junctional complexes. The expression of CAMs has been shown to occur before morphogenetic events (Edelman et al., 1983) and is assumed to play a role in cell-cell adhesion and aggregation (Bertolotti et al., 1980; Edelman, 1983), as well as in morphogenetic and inductive processes (Edelman, 1984; Thiery et al., 1984). In an in vitro model of kidney differentiation, E-cadherin/uvomorulin was found to be expressed early after induction, as soon as epithelial cells begin to aggregate (Saxén et al., 1984), before they acquire junctions (Wartiovaara, 1966). However, in our study, as in previous analyses of in vivo kidney development (Thiery et al., 1984; Vestweber et al., 1985; Klein et al., 1988) E-cadherin/uvomorulin could not be detected in aggregating mesenchymal cells.

At the vesicle stage, the molecules associated with junctional complexes are either absent or undetectable (ZO-1, desmoplakin), or present in small amounts (E- and P-cadherins, plakoglobin). At the S-shape body stage, P-cadherin, plakoglobin and vinculin are clearly expressed by the future glomerular cells, whereas E-cadherin/uvomorulin could not be detected either by us or by Vestweber et al. (1985). This negativity could be due to antigen inaccessibility, or its very low expression.

The plakoglobin immunoreactivity observed at the vesicle and S-shape body stages raises the question as to whether the cells are equipped with intermediate junctions, or desmosomes as suggested by Garrod and Fleming (1990). In the absence of desmoplakin immunoreactivity reproducibly reported by us and others (Aoki, 1967; Bachman et al., 1983; Holthöfer et al., 1984), the existence of desmosomes is quite unlikely.

During the capillary loop stage, future parietal glomerular epithelial cells and podocytes follow strikingly different pathways to gradually acquire the features of mature cells: molecules associated with junctional complexes disappear from future podocytes and are retained by maturing and mature parietal glomerular epithelial cells, as reported by Thiery et al. (1984), Crossin et al. (1985), but not by Vestweber et al. (1985). We detected low levels of vinculin expression at the basal part of podocytes, as previously noted (Drenckhan and Franke, 1988; Schnabel et al., 1990). This immunofluorescence finding could be correlated with the transient presence of interpedicel junctions observed at the ultrastructural level (Saxén and Wartiovaara, 1968; Saxén et al., 1988; Reeves et al., 1978). The nature of these junctions—adherens type (Aoki, 1967) or tight type (Reeves et al., 1978; Minuth et al., 1981)—has been a matter of controversy. More recently, Schnabel et al. (1990) and Kurihara et al. (1992a) described a transient, faint and discontinuous expression of the ZO-1 protein on semi-thin frozen sections of kidneys from developing rats fixed by perfusion. This finding suggests that intercellular junctions between podocytes are of the tight type. However, the presence of ZO-1 protein and of adhesive molecules specific for tight and intermediate junctions has proven difficult to detect. In our study, the absence of ZO-1 protein immunolabeling at any stage of glomerulogenesıs is likely to result from the technique used for fixation. The detection of ZO-1 protein in ureteral buds and ampullary cells is probably due to the stronger expression of the molecule in these epithelial structures. Alternatively, other types of molecules, not yet identified, may be responsible for the increased adhesiveness of cells during the initial steps of glomerulogenesıs.

The expression of one class of intermediate filaments is generally cell type-specific (Franke et al., 1982b; Moll et al., 1982; Osborn et al., 1985) and exclusive (Franke et al., 1978; Sun et al., 1979; Holtzer et al., 1982; Osborn et al., 1982; Waldherr and Schwachheimer 1985; Gröne et al., 1987; Moll et al., 1991). Cells derived from mesenchyme express vimentin (Scheffer et al., 1982; Kasper, 1989; Markl, 1991), while cells of epithelial origin possess cytokeratins (Franke et al., 1978; Sun and Green 1978; Sun et al., 1979, 1983; Franke et al., 1982b). However, there are exceptions: vimentin is not exclusive of mesenchymal cells (Markl, 1991), and cytokeratins have been found in cells of different histogenetic origin (Lane et al., 1983). Two types of intermediate filaments can be expressed in embryonic cells when they switch from one developmental program to another (Erickson et al., 1987; Kasper, 1989; Page, 1989), and in rare cases in normal cells (Wu et al., 1982; Czernobilsky et al., 1985; Kasper et al., 1987; Kasper 1989). In this study, vimentin was the exclusive filament found in uninduced and induced mesenchymal cells of metanephrogenic blastema, as previously reported (Holthöfer et al., 1984; Lehtonen et al., 1985;
Garrod and Fleming, 1990; Oosterwijk et al., 1990; Moll et al., 1991). Conversely, coexpression of vimentin and keratins was observed in typical epithelial cells of ureteral buds and ampullae according to some (Holthöfer et al., 1984; Moll et al., 1991) but not all (Lehtonen et al., 1985; Oosterwijk et al., 1990) previous reports. Both types of filaments were also detected in future podocytes at the S-shape body and capillary loop stages, in agreement with Moll et al. (1991), but not with Holthöfer et al. (1984). Both types were also present in mature parial glomerular epithelial cells, as previously seen by Waidherr and Schwechheimer (1985), Kasper (1989), Moll et al. (1991), but not by Holthöfer et al. (1984) or Cordon-Cardo et al. (1987). Mature podocytes are exclusively vimentin positive, as previously reported (Bachman et al., 1983; Holthöfer et al., 1984; Cordon-Cardo et al., 1987; Moll et al., 1991).

In summary, epithelial cells of ureteral buds and ampullae which possess stable, typical junctional complexes, strongly express their associated molecules and the cytoskeletal components characteristic of epithelial cells. Future glomerular cells at vesicle and S-shape body stages are endowed with transient, atypical junctional complexes and show a low expression of their associated molecules. Podocytes progressively lose CAMs during maturation and, in mature glomeruli, they are characterized by the absence of CAMs and the exclusive expression of vimentin. Therefore, these strictly vimentin positive cells no longer possess classical characteristics of epithelial cells. These observations suggest that a correlation exists between adhesive molecule expression and a stable epithelial phenotype.

In conclusion, the present study shows for the first time that P-cadherin is expressed by future glomerular cells at the initial steps of glomerulogenesis. There is a spatiotemporal correlation between the onset and the loss of expression of P-cadherin and the appearance and disappearance of junctional complexes. It is now of interest to investigate whether there is any renewed expression of junction-associated molecules by dedifferentiated podocytes in nephrotic kidneys. Kurihara et al. (1992b) recently reported the presence of the ZO-1 protein at the level of filtration slits in nephrotic rat glomeruli treated with puromycin aminonucleoside or protamine sulfate. It would therefore be interesting to determine whether cell adhesion molecules such as P-cadherin are reexpressed by the podocytes of nephrotic human or rat kidneys that have similarly treated.

Materials and Methods

Monoclonal antibodies

Mouse monoclonal antibodies to human E- and P-cadherins were the generous gift of Dr. Hirohashi (National Cancer Center Research Institute, Tokyo, Japan). The rat monoclonal antibody to ZO-1 protein (R26.4C) was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA, and the Department of Biology, University of Iowa, Iowa City, IA, USA, under contract NO1-HD-6-2915 from the NICHD. Mouse monoclonal antibodies to desmoplakin and plakoglobin were obtained from Progen (Heidelberg, Germany). Mouse monoclonal antibody directed against human vinculin was from Sigma Chemical Co. (St Louis, MO, USA). Anti-cytokeratin pan K1 was from Immunotech (Hamburg, Germany). Antibody to vimentin was purchased from Amersham (Buckinghamshire, UK). FITC-conjugated goat anti-mouse and anti-\(\beta\)-gal immunoglobulins were obtained from Silenus Laboratories (Hawthorn, Australia). Rhodamine conjugated phalloidin was from Molecular Probes (Inc. Junction City, Oregon, USA).

Tissues

Fetal human kidneys were obtained from seven fetuses (estimated gestational ages: 16, 19, 20, 24, 25, 27 and 38 weeks), autopsied after spontaneous abortions, or abortions of abnormal fetuses performed for medical reasons (Service of Anatomo-Pathology, Pr. Forest, Cochin Hospital, Paris, France). All kidneys were macroscopically normal. Fetal kidneys from Large White pigs were also used. Two fetuses were sacrificed at 40 days, five at 110 days of gestation and one at birth. Five Microgrip strain pig fetuses were also sacrificed at 40 days of gestation.

Immediately after removal, kidneys were cut into small pieces, frozen by immersion in liquid nitrogen and stored at -180°C until used.

Immunofluorescence microscopy

For indirect immunofluorescence microscopy, 3-\(\mu\)m-thick sections of frozen tissues were mounted on glass slides and allowed to air dry at room temperature for 5 min prior to fixation in freshly prepared 3% or 0.3% formaldehyde (wt/vol) in PBS pH 7.2, at room temperature for 20 min. The slides were washed thoroughly with PBS and incubated for 45 min at room temperature with the appropriate antibodies. The primary antibodies were used as follows. Monoclonal antibodies to human E- and P-cadherin were applied at 1:400 dilution. Rat monoclonal R26.4C against ZO-1 protein and mouse monoclonal anti-vinculin were used undiluted. The R26.4C antibody was used only for pig fetuses because it does not cross react with human tissues. Mouse monoclonal antibodies to plakoglobin, desmoplakin and vimentin were diluted 1:5. The ant ipan-cytokeratin K1 antibody was used at 1:2 dilution. The sections were washed 3 times with PBS and incubated with the second antibodies at room temperature for 45 min. The sections were mounted in PBS glycerol containing 0.1% p-phenylenediamine to prevent fading of the fluorescein label during microscopic observation (Platt and Michael, 1983).

Sections to be labeled with rhodamine-phalloidin were fixed with 3% formaldehyde in PBS pH 7.2, under the same conditions as above. They were washed with 3 changes of PBS and incubated with rhodamine-phalloidin (diluted 1:20) in PBS at room temperature for 45 min. The sections were washed and mounted in PBS glycerol as described above. The sections were examined under a Leitz Orthoplan photomicroscope.

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