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Corticosteroid-induced polycystic kidney disease. A morphological study

José L. Ojeda

Department of Anatomy and Cell Biology, University of Cantabria, Spain.

SUMMARY

A single injection of methylprednisolone acetate to the newborn rabbit induces the development of polycystic kidney disease (PKD). In a first stage collecting duct cysts (CDCs) develop. Enlargement of ducts and CDC formation are accompanied by preservation of normal principal to intercalated cell ratios and modifications in the structure and composition of the CDC basement membrane. These changes appear at the beginning of tubular dilation, are not observed in other renal basement membranes, and disappear progressively during regression of the CDCs. It is likely that an abnormal basement membrane modifies the spatial and chemical signals encoded within the extracellular material, which, in turn, could lead, via integrins, to abnormal control of the size of the collecting duct, which then undergoes cystic dilation.

Glomerular cyst formation occurs in later stages and is accompanied by CDC regression. Glomerular cysts are unique in that the parietal epithelial layer undergoes transformation to podocytes (parietal podocytes). This transformation occurs in the absence of capillaries. Parietal podocytes provide a new tool for the study of glomerular epithelial differentiation, the functional capacity of isolated podocytes in vivo, and the assembly of the glomerular filtration surface.

Key Words: Cystic disease - Corticoids - Parietal podocytes - Glomerular basement membrane - Extracellular matrix.

INTRODUCTION

The presence of enormous dilated segments of nephrons or collecting ducts is the central feature of polycystic kidney disease (PKD). The cysts give the kidney a characteristic sponge-like appearance. The progressive development and enlargement of numerous fluid-filled cysts in the kidney ultimately lead to renal failure, which requires chronic dialysis or renal transplantation for patient survival. Cysts can also develop in other organs, particularly the liver and there is a significant increase in connective tissue disorders associated with PKD.

The prevalence of PKD ranges from 1:500 to 1:1,000 and it is seen in 10 to 12 per cent of all patients with end-stage kidney disease. In comparative terms, the incidence and social costs of PKD are greater than those related to hemophilia.

Despite intensive genetic (Harris, 1996) and molecular (Peters et al., 1996) investigations, the pathogenesis of PKD remains unknown. In essence, the cysts are giant segments of nephrons or collecting ducts that, initially at least, retain both their structure and function (Grantham, 1983). This implies that in PKD there is an alteration in the mechanisms controlling tubular size, size being a basic component of organic form.

Analysis of PKD may not only provide information about its pathogenesis, but may also be of value in the study of factors controlling normal kidney development. In this review, special attention will be paid to the latter aspect.

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Correspondence to:
Prof. José L. Ojeda. Departamento de Anatomía y Biología Celular. Facultad de Medicina.
C/ Cardenal Herrera Oria, s/n. 39011-Santander, Spain.
E-mail: ojedajl@medi.unican.es

Models of cystic kidneys

The analysis of PKD requires the development of experimental models in which the disease can be induced easily and consistently, thus allowing longitudinal assessment and adequate description of the course of the disease. At present, there is a number of animal models for PKD (for a review, see: Avner et al., 1990). Both in vivo and in vitro models have been used to study the pathogenesis of renal cyst formation. In vivo studies in a number of animal species have focused on the induction of cystic disease by chemical cystogens or by surgical manipulation and on the analysis of genetically transmitted PKD. In vitro studies have focused on the production of renal cysts in renal organ cultures, and in cell cultures of epithelia from animal and human PKD.

The corticosteroid model

Since Baxter's (1960) original description of cortisone-induced renal cystic changes in rabbits, several corticosteroids have been used to induce PKD in different mammalian species (for a review, see Avner et al., 1990). Here we review the main results from the study of corticoid-induced PKD in the rabbit.

PKD is induced in the newborn rabbit by a single intramuscular injection of methylprednisolone acetate (20 mg/kg body weight). Renal cysts develop in 100 per cent of rabbits. Renal microdissection and light and electron microscopic analyses have revealed three distinct stages of cyst development (Ojeda et al., 1972). There is an initial stage of renal immaturity, with the persistence of the postnatal subcapsular metanephrogenic zone and dilation of the terminal ampullae of the collecting tubules. Abnormal cell death with regard to both location and intensity is observed at this stage (García-Porrero et al., 1978). Apoptosis has been suggested to be an important factor in the evolution of the PKD lesions, and probably also in the initiation of the pathological process (for a review, see Zhou and Kukes, 1998). In the second stage, collecting duct cysts (CDCs) (Figs. 1 and 2) reach their maximum size (up to 400 μm in transverse diameter). A characteristic feature of this stage is the presence of developing glomerular cysts. In addition, some proximal tubules display dilations and modifications of their convolutions (Ojeda and García-Porrero, 1981). In the third stage, glomerular cysts (Fig. 2 inset) are fully developed. They are spherical, contain one or more small glomeruli, and consist of a dilation of the urinary space (Bowman's space). Glomerular cyst enlargement is accompanied by regression of the CDCs, which progressively return to normal size (Ojeda et al., 1990). Renal cyst involution has also been reported in other animal PKD models (Avner et al., 1989; Carone et al., 1994) and in human polycystic kidneys during replacement therapy (Thaysen and Thomsen, 1982) or after chronic hemodialysis (Ishikawa et al., 1984). Cyst regression underscores the enormous plasticity of adult renal tissues to control and maintain their normal structure and opens up the possibility of a cure for PKD.

Collecting duct cysts

Transmission and scanning electron microscopy and lectin labeling observations show that the epithelium of CDCs comprises the two main normal cell populations, principal and intercalated cells (Fig. 3), and that the ratio, distribution and types of intercalated cells are similar in normal collecting ducts and CDCs (Ojeda et al., 1986). These findings support the hypothesis that renal cyts are giant ducts which conserve both the morphology and the function of the epithelium. Furthermore, since intercalated cells differentiate in the rabbit during postnatal life and no differences are observed in the time of the appearance of these cells between normal and cystic collecting ducts, their development seems to be independent of both corticoid effects and cystic changes (Ojeda et al., 1986).

The most striking morphological changes displayed by CDCs are the structural and compositional modifications to their basement membrane (Ojeda et al., 1987). These modifications appear as the collecting duct begins to dilate and are not observed in other renal basement membranes. The basement membrane of CDCs is seen to be thickened and multilayered (Fig. 4), with numerous matrix vesicles similar to those described in tissues incalcification (Ojeda et al., 1990). The amorphous material surrounding the CDCs is intensely stained by ruthenium red (Fig. 5). Immunofluorescent staining for laminin and type IV collagen appears slightly decreased in the basement membrane of CDCs. In contrast, the amount of fibronectin is clearly increased (Ojeda et al., 1990). Modifications in glycoproteins of the extracellular material seem to be a common feature in corticoid-induced PKD. Thus, in the murine autosomal recessive PKD that courses with elevated corticosterone levels (Crocker et al., 1987), the abnormal presence of the oncofetal glycoprotein tenascin has been detected in the CDC basement membrane (Ojeda, 1999).

During the regression of CDCs, the basement membrane abnormalities progressively disappear. The early onset of these basement membrane modifications, their selective nature, and their disappearance with CDC regression suggest that there is a direct relationship between these alterations and CDC development. Alterations in the extracellular materials seem to be a constant fea-

ture in PKD, since changes similar to those observed in corticosteior-induced PKD have been described in human PKD (Milutinovic and Agodoa, 1983) as well as in different animal PKD models (Carone et al., 1994; Calvet, 1993). Furthermore, the discovery by the European Polycystic Kidney Disease Consortium (1994) that the PBP (polycystic breakpoint product) gene product may be an extracellular material protein supports the hypothesis that the immediate cause of PKD would be alteratons in extracellular materials. However, the mechanism by which changes in extracellular materials result in the development of CDCs is still unclear.

There is little to support the idea that abnormal compliance of the tubular basement membrane is a primary event in the development of renal cysts. Young's modulus of a basement membrane depends, among others factors, on its thckness (Welling and Grantham, 1972). Therefore, a thickened basement membrane would prevent the ballooning of the tubules. Also, the simple ballooning of a renal tubule segment is not compatible with the normal morphology of the cystic cells (Grantham, 1983). Finally, the development of renal cysts is not accompanied by changes in the viscoelastic properties of the basement membrane (Grantham et al., 1987).

Increasing evidence accumulated over the last 10 years has indicated that the extracellular materials provide instructive information to the cell and modulate gene expression, mainly via integrins (for review see: Boudreau and Bissell, 1998). Thus, changes in the composition and structure of the basement membrane may modify the signals received by renal cells, inducing the epithelium to develop abnormally and resulting in the formation of cystic structures.

The close association between the alterations in extracellular materials and the development of giant segments of the collecting tubules suggests that the size of these tubules, and possibly the size of the different nephron segments, are controlled by signals encoded in the extracellular materials, among others factors.

Glomerular cyst

Glomerular cysts are unique in that the parietal epithelial layer undergoes transformation to podocytes (Ojeda et al., 1979). Accordingly, these cells have been termed parietal podocytes (PPs) (Fig. 6) (Ojeda and García-Porrero, 1982). PPs are the same size and shape as normally situated podocytes (visceral podocytes), including interdigitating foot processes (Fig. 6 inset). Furthermore, the pattern of lectin staining is the same as for visceral podocytes (Ojeda et al., 1993). The presence of PPs is not exclusive to this experimental model since they have also been described in renal cysts in macaques (Miyoshi et al., 1984) and in a number of human renal diseases (Pardo-Mindan et al., 1978; Gibson et al., 1992).

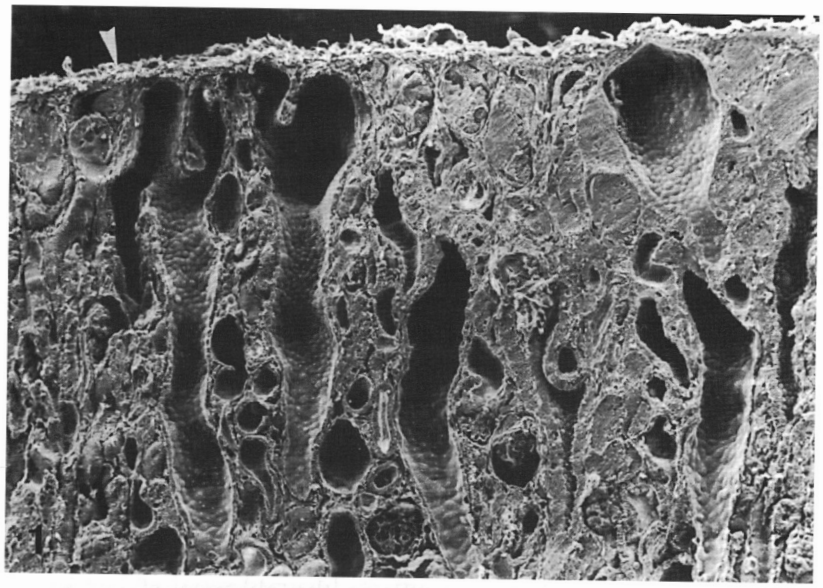
The developmental sequence in the genesis of PPs is similar to that reported for visceral podocytes and includes a metaplastic transformation of the parietal cells of Bowman's capsule. This transformation occurs without the presence of capillaries (Fig. 7). It has been suggested that the development and differentiation of podocytes depends on an endothelial factor (Vernier and Birch-Andersen, 1962) and that the differentiation of the glomerular capillaries precedes that of the podocytes (Hay and Evan, 1979). However, the development and differentiation of PPs in the absence of capillares (Ros et al., 1985) clearly show that the hypothesis of endothelial control of podocyte development is not correct. This conclusion is in accordance with results for mouse metanephros in culture (Bernstein et al., 1981).

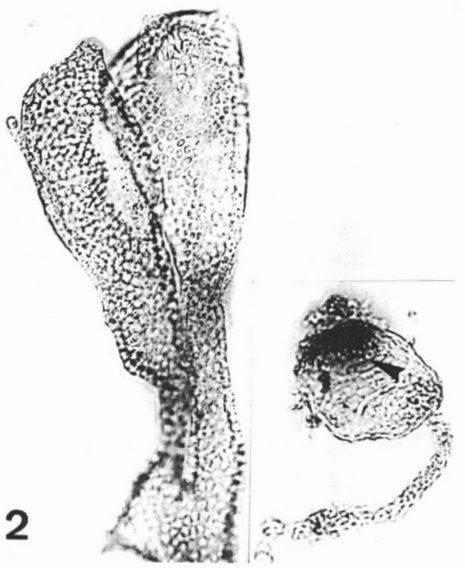
Unlike CDCs, PPs do not display a thickening or other morphologic alteratons of the basement membrane (Fig. 7) (Ojeda et al., 1989). Thus, the development of glomerular cysts does not seem be due to modificatons of the extracellular material surrounding the cysts. The dilation of Bowman's capsular space may be explained by the fact that the area occupied by the PPs with their many long ramifications in much greater than the area that the same number of normal parietal cells would occupy, thus creating an incongruity in the relative size of Bowman's capsule and the glomerulus.

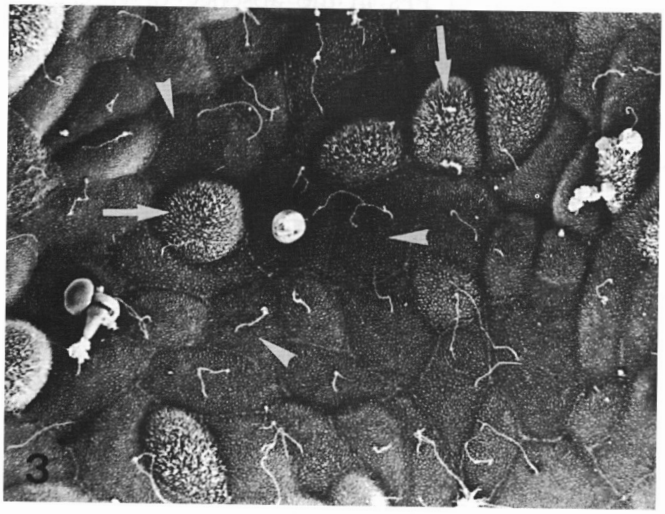
PPs provide a new tool for the study of glomerular epithelial differentiation, the functional capacity of the isolated podocytes in vivo, and the assembly of the glomerular filtration surface.

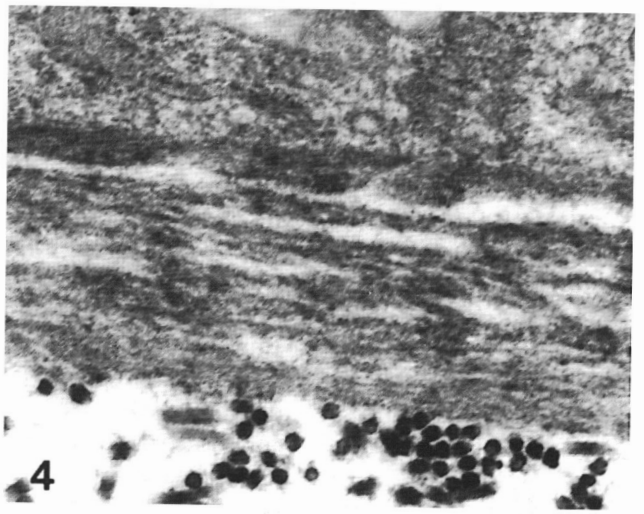
Intravascular injection of electron-opaque tracers with different charges and molecular sizes (ferritin, horseradish peroxidase, and cationic colloidal iron) has allowed the study of the functional behavior of PPs. After passing through the glomeruli, the tracers cross the urinary space, pass through the PP filtration slits and finally label their basement membrane (Fig. 8). Tracer experiments demonstrate that during their passage through the filtration slits, the molecules are not polarized by the slit diaphragms, since the tracers may pass in the opposite direction. This algo demonstrates that the glomerular cysts preserve their functional capacity.

The structural and chemical composition of the glomerular basement membrane (GBM) is a major determinant of glomerular filtration (Kanwar, 1984). At least two cell types are involved in GBM assembly: podocytes and the endothelial cells of the glomerular capillaries (Abrahamson, 1985). The GBM exhibits certain special characteristics within the kidney. It has a complex structure (Abrahamson, 1987), responds in uni-









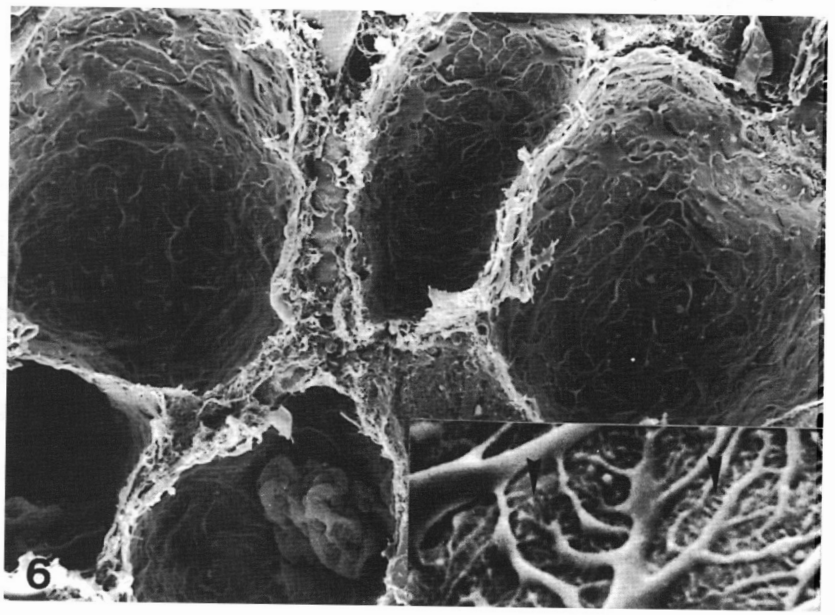


Fig. 1.– SEM micrograph of a factured polycystic kidney from a 15-day-old animal. Note the spongy appearance of the outer renal cortex and the wide communication that the collecting duct cysts establish with the rest of the tubular system. Arrowheads, renal capsule. x 160.

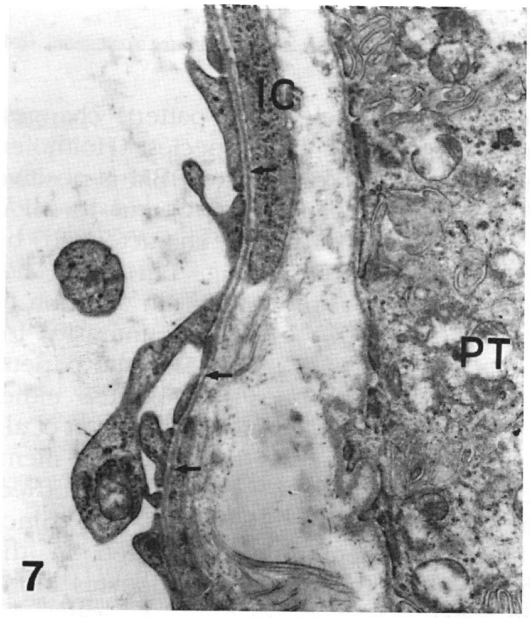
Fig. 2.– Microdissection of two collecting duct cysts and one glomerular cyst (inset). Arrowhead, glomerulus. Phase-contrast. x 170.

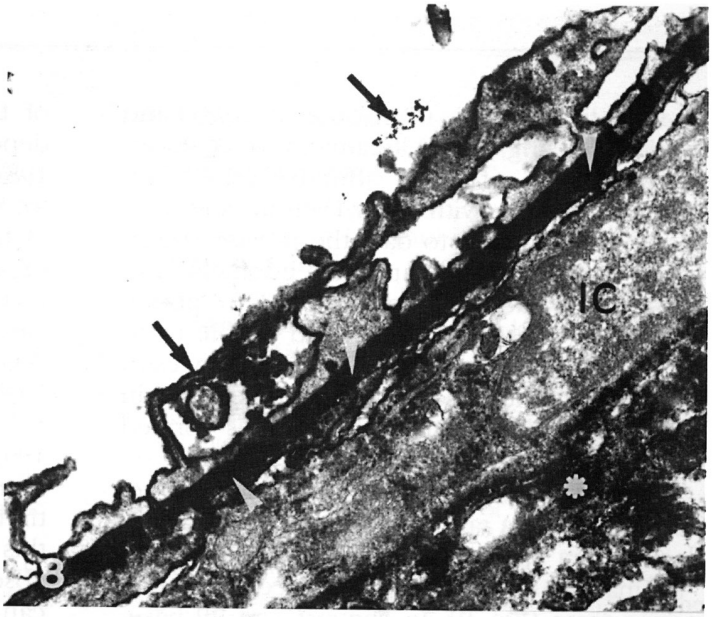
Fig. 3.– SEM micrograph showing principal (arrowheads) and intercalated (arrows) cells in a collecting duct cyst. x 1,200.

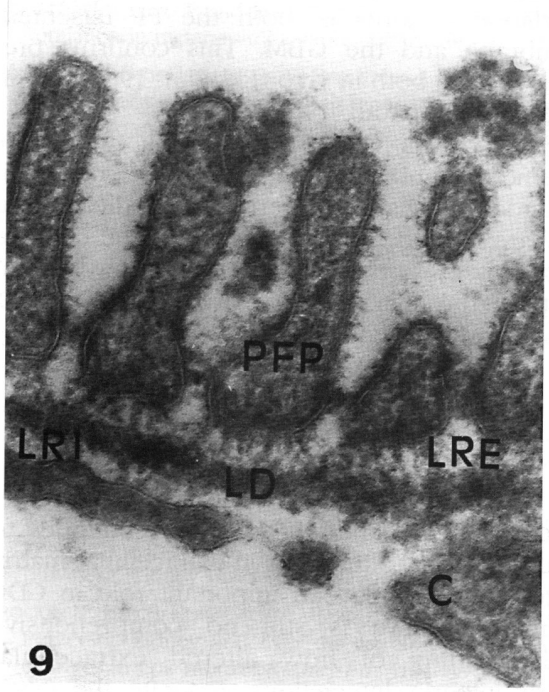
Fig. 4.– TEM micrograph showing the thickened basement membrane of a collecting duct cyst. The basement membrane appears formed by numerous layers of electron-dense material. Tannic-acid fixation. x 40,000.

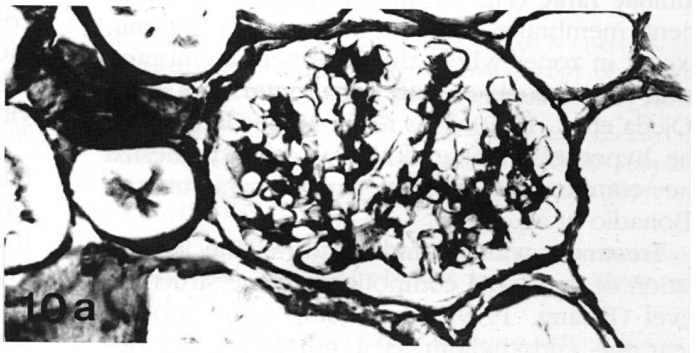
Fig. 5.– TEM micrograph showing the distribution of anionic sites in the multilayered basement membrane of a collecting duct cyst. Ruthenium red fixation. x 40,000.

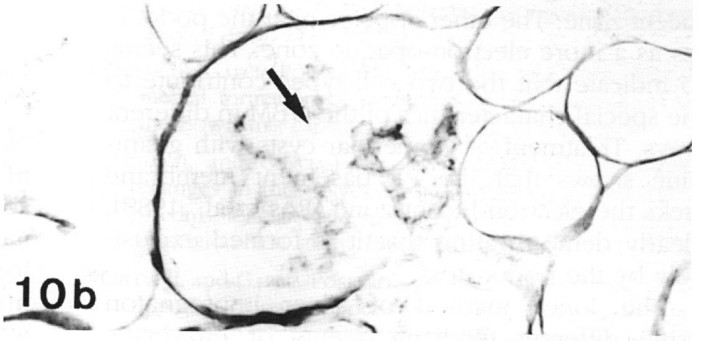
Fig. 6.– SEM micrograph of five fractured glomerular cysts. The parietal layer is fully occupied by podocytes. x 320. Inset: detailed view of the parietal podocytes. The filtration slits can be observed (arrowheads). x 1,300.

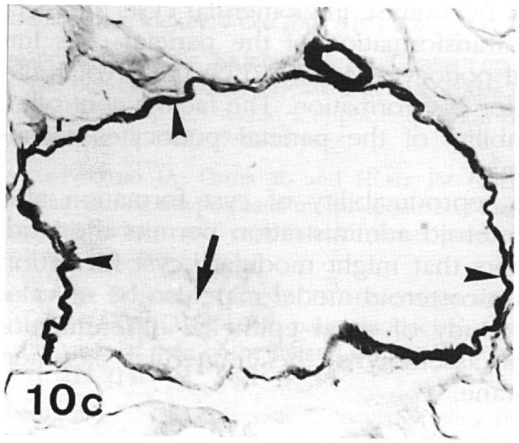


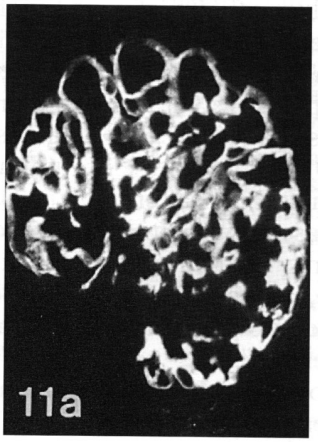












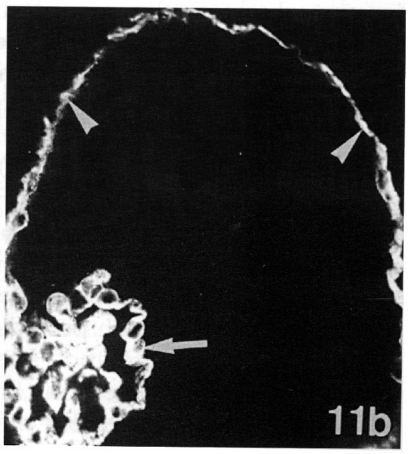


Fig. 7.– TEM micrograph of the parietal layer of a glomerular cyst displaying parietal podocytes. Note that the PP basement membrane (arrows) shows a normal structure and that no capillaries can be seen. IC, interstitial cells; PT, proximal tubule. x 20,000.

Fig. 8.– Horseradish peroxidase distribution in a segment of the parietal layer of a glomerular cyst from an animal sacrificed 10 minutes after tracer injection. Horseradish peroxidase molecules are seen along the PP basement membrane (arrowheads), in the urinary space (arrows), and in the interstitial spaces (asterisk). IC, interstitial cell. x 15,000.

Fig. 9.– TEM micrograph showing the normal structure of the GBM. PFP, podocyte foot process; C, capillary; LD, lamina densa; LRE, lamina rara externa; LRI, lamina rara interna. Tannic-acid fixation. x 80,000.

Fig. 10.– Sections of normal renal corpuscles **(a, b)** and of a glomerular cyst **(c)** silver-stained according to Jones method. Using periodic acid as oxidizing agent **(a)** all basement membranes of the renal corpuscle are stained. After lysozyme digestion **(b, c)**, the GBM appears unstained (arrows); however the PP basement membrane shows an intense silver affinity (arrowheads). x 600.

Fig. 11.– Fluorescence micrographs of a normal renal corpuscle **(a)** and of a glomerular cyst **(b)** stained with FITC-conjugated lectin PNA after neuraminidase treatment. Note that in the normal renal corpuscle only the GBM appears positive for PNA, whereas in the glomerular cyst both the GBM (arrow) and PP basement membranes (arrowheads) appear intensely PNA-positive. x 400.

que ways to enzymatic (Carlson et al., 1981) and chemical (Huang, 1979) treatment, and shows special patterns of lectin affinities (Holthöfer, 1983) and staining with silver (Velican and Velican, 1970). However, to date the precise contributions of the podocyte and the endothelium to the special characteristics of the GBM are poorly understood. The PP model should enable us to precisely define the contribution of podocytes to the formaton of its basement membrane, since in glomerular cysts Bowman's capsule is formed only by podocytes.

The GBM presents a typical trilaminar structure with a lamina rara externa near the pedicels, a lamina rara interna near the endothelium, and a lamina densa interposed between the two laminae rarae (Fig. 9). In contrast, the PP basement membrane lacks a lamina rara interna, except in zones where this basement membrane is in close contact with renal interstitial cells (Ojeda et al., 1989). This result argues in favor of the hypothesis that endothelial cells synthesize the components of the lamina rara interna (Bonadio et al., 1984).

Treatment with guanidine allows the identfication of two GBM components at the structural level (Huang, 1979). One component appears near the endothelium as a relatively electron-lucent zone. The other appears near the podocytes as a more electron-opaque zone. This seems to indicate that the two cell types contribute to the special characteristics of the GBM in different ways. Treatment of glomerular cysts with guanidine shows that the PP basement membrane lacks the electron-lucent zone (Ros et al., 1988), clearly demonstrating that it is formed exclusively by the podocytes.

The Jones method of silver impregnaton using different oxidizng agents or enzymatic digestion discloses the special silver affinity of the different basement membranes of the renal corpuscle (Fig. 10 a-c) (Velican and Velican, 1970), which may be due to the special way it is formed. The Jones method shows that whereas the GBM of both normal (Figs. 10 a, b) and cystic glomeruli exhibit silver affinity only after periodic-acid oxidation, the PP basement membrane exhibits the same silver affinity as the normal parietal basement membrane, a deep-black color appearing after both periodic-acid and permanganate oxidation, and after elastase or lysozyme (Fig. 10 c) digestion (Ros et al., 1988). Thus, although this basement membrane is formed only by podocytes, its silver affinity is clearly different from that of the GBM. This suggests that the lack of silver affinity of the GBM after permanganate-acid oxidation or after different enzymatic digestions is due to its endothelial component.

The lectin-binding pattern allows the characterization of the different basement membranes of the renal corpuscle. This pattern changes, depending on the animal species (Holthöfer, 1983). In the rabbit kidney, the GBM is positive to WGA, to succinylated WGA, and to MPA. Maturation of the GBM is characterized by expression of both PNA (Fig. 11a) and MPA cryptic-binding sites. In contrast, in addition to clear differences in the staining intensity for WGA, succinylated WGA and MPA, the parietal basement membrane does not express either PNA (Fig. 11a) or MPA cryptic sites (Ojeda et al., 1993). In glomerular cysts, the basement membrane of the PP shows the same pattern of lectin-binding sties as the GBM (Fig. 11b). Since this basement membrane is formed in the absence of endothelium, the podocytes alone can be considered to be responsibe for the glycosylation patterns of both the PP basement membrane and the GBM. This confirms previous results, both in vivo (Ekblom, 1981) and in vitro (Bonado et al., 1984).

Finally, it is worth emphasizing that cystic basement membranes show normal morphological and glycosylation patterns. This indicates that they are functional structures, since the charged glycoproteins of the GBM are the major determinants of the characteristics of glomerular filtration (Kanwar, 1984).

Conclusion

In conclusion, although evidence continues to accumulate that alterations in extracellular matrix molecules may play an important role in CDC formation, there is still no comprehensive understanding of how altered extracellular matrix/integrin interactions generate the cystic lesions. In contrast, in glomerular cysts the metaplastic transformation of the parietal cells into parietal podocytes seems to be the pivotal alteration for cyst formation. The factors controlling the stability of the parietal podocytes remain unknown.

The reproducibility of cyst formation after corticosteroid administration permits the study of factors that might modulate cyst formation. The corticosteroid model may also be of value in the study of renal epithelial differentiation and the assembly of the glomerular basement membrane.

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