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Cell type-specific acquired protection from crystal adherence by renal tubule cells in culture

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Cell type-specific acquired protection from crystal adherence by renal tubule cells in culture.

Background. Adherence of crystals to the surface of renal tubule epithelial cells is considered an important step in the development of nephrolithiasis. Previously, we demonstrated that functional monolayers formed by the renal tubule cell line, Madin-Darby canine kidney (MDCK), acquire protection against the adherence of calcium oxalate monohydrate crystals. We now examined whether this property is cell type specific. The susceptibility of the cells to crystal binding was further studied under different culture conditions.

Methods. Cell-type specificity and the influence of the growth substrate was tested by comparing calcium oxalate monohydrate crystal binding to LLC-PK_1 cells and to two MDCK strains cultured on either permeable or impermeable supports. These cell lines are representative for the renal proximal tubule (LLC-PK_1) and distal tubule/collecting duct (MDCK) segments of the nephron, in which crystals are expected to be absent and present, respectively.

Results. Whereas relatively large amounts of crystals adhered to subconfluent MDCK cultures, the level of crystal binding to confluent monolayers was reduced for both MDCK strains. On permeable supports, MDCK cells not only obtained a higher level of morphological differentiation, but also acquired a higher degree of protection than on impermeable surfaces. Crystals avidly adhered to LLC-PK_1 cells, irrespective of their developmental stage or growth substrate used.

Conclusions. These results show that the prevention of crystal binding is cell type specific and expressed only by differentiated MDCK cells. The anti-adherence properties acquired by MDCK cells may mirror a specific functional characteristic of its *in situ* equivalent, the renal distal tubule/collecting ducts.

Renal stones are largely composed of calcium salts that are precipitated from the tubular fluid. The crystallization of stone salts results from the ability of the kidney to concentrate the tubular fluid [1]. The selective reabsorption of water and solutes takes place in distinct segments of the nephron [2]. The distribution of these reabsorptive capacities is reflected in different risks for crystallization at the various sites in the nephron [3–7]. The first nucleation risk, for example, is encountered in the loop of Henle, which disappears as the fluid becomes diluted in the ascending limb and distal tubule [3–7]. During antidiuresis, extensive net water reabsorption leads to an increased risk for crystal formation in the collecting duct toward the papillary tip [4–8]. Crystals formed in the tubular fluid are not necessarily harmful as long as they are eliminated with the urine. Crystal growth and agglomeration, however, could create particles that are too large to freely pass the renal tubules [9–11]. In addition, cell culture studies [12–14] have indicated that crystals can bind to the surface of the renal tubule epithelium. Indeed, crystal deposits with a size smaller than the diameter of the tubules have been found attached to the luminal surface of renal tubular cells in hyperoxaluric patients [15, 16] and rats [17].

Previously, we showed that the affinity of the luminal surface of MDCK-I cells for calcium oxalate monohydrate (COM) crystals highly depends on the developmental stage of the cultures. Relatively large amounts of crystals could adhere to subconfluent cultures, whereas intact and functional monolayers were largely protected from crystal binding [18]. It was speculated that the anti-adherence capacity of functional MDCK cultures may reflect a physiologically relevant property. If so, we expect anti-adherence properties to be present in cell types that are frequently confronted with crystals and absent in cells that normally do not encounter crystals. Indeed, calcium oxalate crystals did not adhere to the surface of the transitional epithelium of the bladder [19], but were found to bind avidly to the surface of red blood cells [20], leukocytes [21], endothelial cells [22], and fibroblasts [23], which are normally not exposed to such crystals. Based on the literature, calcium oxalate crystals

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are frequently present in the tubular fluid of the renal collecting duct, but not in that passing the proximal tubule. To test this hypothesis further, we extended the crystal-binding studies to include another cell line with characteristics of the renal collecting duct (MDCK-II), as well as a cell line that closely resembles the renal proximal tubule (LLC-PK_1). Because the development of cultured cells is influenced by the substrate on which they are grown, these experiments were performed with cells grown on permeable and impermeable surfaces to study the relationship between the developmental stage of the cells and crystal binding more closely.

METHODS

Cell culture

Madin-Darby canine kidney-I [24] and MDCK-II [25] were kindly provided by Professor G. van Meer (Laboratory for Cell Biology and Histology, Amsterdam Medical Center, Amsterdam, The Netherlands). LLC-PK_1 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were routinely seeded at a plating density of 2.2×10^5 cells/cm^2 on either polycarbonate porous filter inserts (Transwells, 24 mm, 0.4 μm pore size) or on tissue culture plates (12 wells, 24 mm; both obtained from Corning Costar, Badhoevedorp, The Netherlands). The cells were cultured in Dulbecco's modified minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS). Culture medium was refreshed every other day. The cell lines were routinely checked for mycoplasma contamination and were found to be negative in all experiments described in this study. Transepithelial electrical resistances were measured with an EVOM-G connected to an Endohm-24 (World Precision Instruments, Sarasota, FL, USA).

Preparation of calcium oxalate crystal suspension

A solution of radioactive sodium oxalate was prepared by adding 1 ml 0.37 MBq/ml [^{14}C]oxalic acid (Amersham, Buckinghamshire, UK) to 0.25 ml 200 mM sodium oxalate. A calcium chloride solution was prepared by adding 0.25 ml 200 mM calcium chloride to 8.5 ml distilled water. After mixing the two solutions at room temperature, COM crystals formed immediately. The suspension was allowed to equilibrate for three days and, after washing, was resuspended in 5 ml CaOx saturated distilled water. Crystal suspensions were kept at room temperature and were freshly prepared every other week.

Calcium oxalate monohydrate crystal binding

The assay to measure COM crystal binding was described previously [18]. The filters were cut out, and the culture dishes were scraped. The level of crystal binding was calculated from the amount of associated dpms and was expressed in μg/cm^2.

Brush border enzyme measurements

Cell monolayers were washed with phosphate-buffered saline (PBS) scraped in 1 ml 50 mM tris(hydroxymethyl)aminomethane (Tris-HCl; pH 7.4) and sonicated for 30 seconds on ice. After centrifugation for 10 minutes at 2000 *g*, the supernatant was used to measure enzyme activity. γ-Glutamyl transpeptidase (γ-GT, EC 2.3.2.2) activity was determined spectrophotometrically, using L-γ-glutamyl-3-carboxy-4-nitroanilide as substrate, and alkaline phosphatase (AP; EC 3.1.3.1) activity was assayed with sodium p-nitrophenylphosphate as substrate.

Na^+,K^+-ATPase polarization

[^{3}H]ouabain (specific activity 20.5 Ci/mmol; New England Nuclear, Boston, MA, USA) was used to quantitate the number of apical and basolateral membrane binding sites [26]. Because the basolateral membrane of cells grown on solid substrates is not accessible, these studies were limited to cells cultured on permeable supports. Total [^{3}H]ouabain binding (0.05 μM) and nonspecific binding in an excess of cold ouabain (50 μM) was measured in a binding buffer containing 1 μCi/ml [^{3}H]ouabain in K^+-free Ringer buffer that consisted of (in mM) 140 NaCl, 1.8 $CaCl_2$, 5 D-glucose, and 10 Tris-HCl, pH 7.4. The cells were washed twice with PBS, and then 1.0 ml binding buffer (37°C) was added at the apical or basolateral side of the monolayers. After 20 minutes, the binding buffer was removed. The cells were washed 10 times in ice-cold Ringer's buffer to which 100 mM $MgCl_2$ was added. The filters were cut out with a scalpel, and radioactivity was counted in a scintillation counter. The nonspecific binding of radiolabeled ouabain was generally lower than 1%. The amount of ouabain binding was calculated and expressed as fmol/10^6 cells.

Confocal laser scanning microscopy

The cells were fixed in 3.7% formaldehyde for 15 minutes and were then permeabilized for 15 minutes with 70% ethanol. Subsequently, the cells were washed with PBS, incubated for 15 minutes with 1 μg/ml fluorescein isothiocyanate-conjugated phalloidin (FITC-phalloidin) at the apical site, washed 2 × three minutes with PBS, and mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA). Images were made with a Zeiss LSM 410 laser scanning confocal microscope (Zeiss, Oberkochen, Germany). A 488 nm Ar-laser was used to excitate the FITC-phalloidin. COM crystals were detected by their reflection of the 633 nm (red) Kr-laser. The FITC emission signal and the 633 nm signal reflected by the crystals were separated by a 560 nm beam splitter. The FITC signal was passed through a 510 to 540 nm band-pass filter to block reflection from the 488 nm laser. No blocking filter was used for the reflection signal.

Scanning electron microscopy

To perform scanning electron microscopy, the cells were fixed in 2.5% glutaraldehyde in cacodylate buffer. After washing, the samples were postfixed in OsO_4 in cacodylate buffer, washed again in cacodylate buffer, dehydrated in a graded series of ethanol, and finally critical point dried. After mounting on stubs, a conductive layer was sputtercoated on the samples and examined in a JEOL JSM 25 scanning electron microscope (JEOL, Tokyo, Japan).

RESULTS

Cell culture characteristics

When cells were grown to confluence on 24 mm permeable filter inserts or 24 mm solid plastic wells, the monolayers always reached a higher cell density on the porous supports. On inserts, MDCK-I, MDCK-II, and LLC-PK_1 became confluent at 3.3 ± 0.3, 3.9 ± 0.4, and $3.9 \pm 0.2 \times 10^6$ cells, respectively, whereas confluency on plastic wells was reached at 2.4 ± 0.4, 3.3 ± 0.2 and $1.9 \pm 0.4 \times 10^6$ cells, respectively. Thus, apparently the cells are more densely packed on permeable growth substrates. The transepithelial electrical resistances of confluent monolayers of MDCK-I, MDCK-II, and LLC-PK_1 cells grown on porous supports were 8200 ± 3500, 70 ± 15, and $98 \pm 15\ \Omega \cdot cm^2$, respectively. To determine whether the cell lines formed polarized monolayers on permeable substrates, the binding of [^{3}H]ouabain to Na^+,K^+-ATPase was measured at the apical and basolateral plasma membrane of confluent monolayers. These studies demonstrated an asymmetrical distribution of Na^+,K^+-ATPase with a predominant localization at the basolateral membrane in all three cell lines (Fig. 1). The level of proximal tubule brush border enzyme (γ-GT and AP) activities was very high in LLC-PK_1 cells, but was almost undetectably low in MDCK-I cells. MDCK-II cells expressed low but measurable AP activities, whereas this cell line expressed intermediately high levels of γ-GT activity (Table 1).

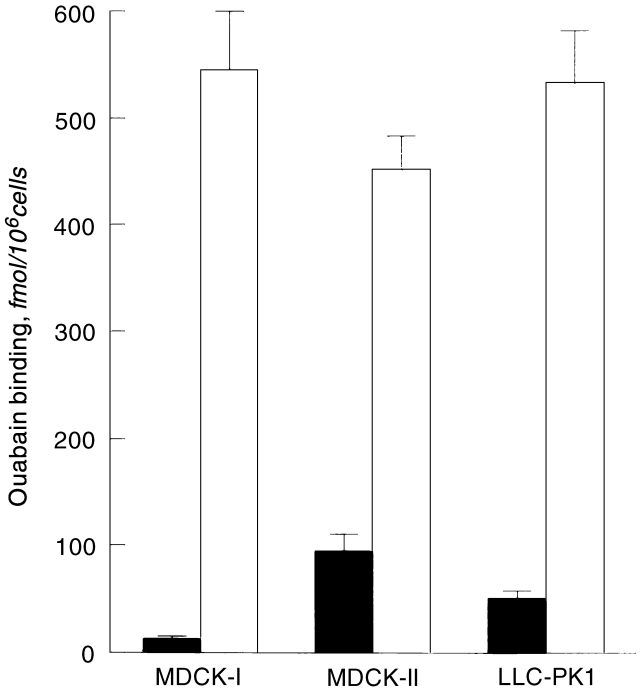


Fig. 1. Plasma membrane distribution of ouabain binding sites. The apical (■) or basolateral (□) membranes of cells grown on pairs of filters for seven days were incubated with radiolabeled ouabain. The amount of specific binding was determined and expressed in fmol/10^6 cells. The results show that all three cell lines formed polarized monolayers. Little ouabain binding was detected on the apical membrane, whereas substantial amounts of binding were detected at the basolateral membrane. The results are representative for three experiments.

Table 1. γ-glutamyl transpeptidase (γ-GT) and alkaline phosphatase (AP) enzyme activity (U/mg protein) in homogenates of filter-grown confluent monolayers of MDCK-I, MDCK-II and LLC-PK_1 cells

	MDCK-I	MDCK-II	LLC-PK_1
γ-GT	2.0 ± 1.5	323 ± 10	993 ± 45
AP	0.2 ± 0.2	3.7 ± 0.2	331 ± 12

Calcium oxalate monohydrate crystal binding

The binding of COM crystals to cells cultured on permeable and impermeable supports is depicted in Figure 2. Two days after seeding, the level of crystal binding to MDCK-I and MDCK-II, as well as to LLC-PK_1, cells was relatively high, irrespective of the growth substrate on which they were cultured. After MDCK-I cells had formed confluent monolayers, the level of crystal binding decreased. Compared with cells grown on impermeable surfaces, the reduction of the crystal-binding capacity was substantially greater in cultures on permeable supports, and in fact, crystal binding was nearly undetectable. A time-dependent decrease in crystal binding was also observed with monolayers formed by MDCK-II cells. On porous supports, these levels did not become as low as those to MDCK-I cells, and relatively high levels of crystal binding were measured to confluent MDCK-II monolayers on impermeable supports. There was no significant decrease in crystal binding to LLC-PK_1 cells cultured on either growth substrate during their growth to confluency (Fig. 2).

Morphological studies

The adherence of COM crystals to confluent monolayers, as well as the morphology of the cells, was visualized by confocal microscopy. These studies revealed that there was a remarkable difference in the morphology of MDCK cells cultured on permeable or impermeable supports (Fig. 3). On permeable substrates, the cells

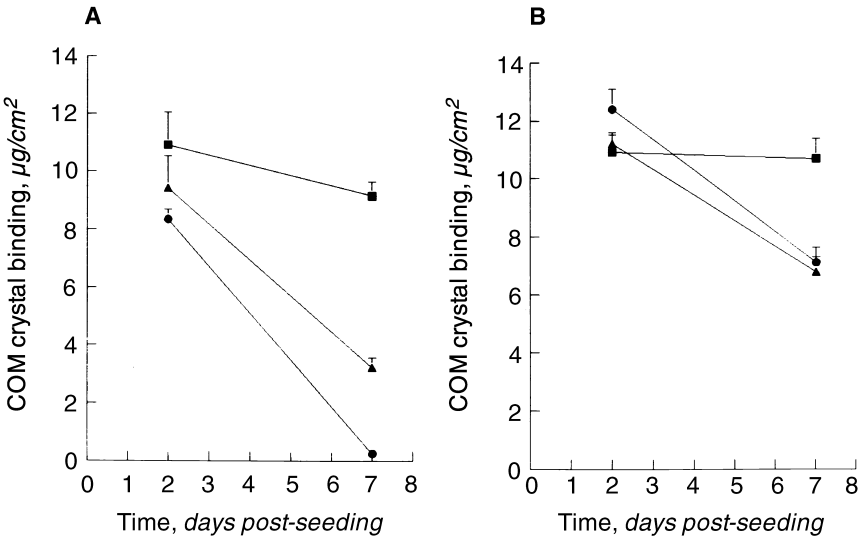


Fig. 2. Binding of crystal oxalate monohydrate (COM) crystals at the surface of cells cultured on permeable (*A*) or impermeable (*B*) growth substrates. Crystal binding was studied two- and seven-days postseeding. The apical membrane received radiolabeled COM crystals (16 μg/cm²) that were allowed to adhere for one hour. The level of crystal adherence was calculated from the amount of surface-associated dpm and was expressed in μg/cm². The results, representative for five experiments, show that both MDCK cell strains cultured on either substrate acquire protection from the adherence of crystals in time. The strongest anti-adherence properties are obtained by MDCK cells cultured on permeable supports. On permeable supports, MDCK-I cells acquire higher levels of protection than MDCK-II cells. Monolayers formed by LLC-PK$_1$ cells on either growth substrate acquire no or hardly any protection against the adherence of crystals.

formed a tight row of cuboidal cell (Fig. 3 C, E), resembling the morphology of its *in situ* equivalent, the collecting duct [27], whereas cells cultured on a solid substrate had a more slender, less differentiated appearance (Fig. 3 D, F). Morphological differences between LLC-PK$_1$ cells cultured on either substrate were less conspicuous. On both substrates, the cells were rather flat (Fig. 3 A, B), which is in agreement with the morphology of the epithelium in the renal proximal tubule [27]. These images further showed that COM crystal adherence is abundant to the surface of LLC-PK$_1$ cells cultured on permeable or impermeable growth substrates (Fig. 3 A, B), usually less abundant to MDCK-II cells on either substrate (Fig. 3 C, D), limited to MDCK-I cells cultured on a solid substrate (Fig. 3F) and absent at the surface of MDCK-I cells cultured on a permeable surface (Fig. 3E).

Scanning microscopy images of filter-grown confluent monolayers showed that LLC-PK$_1$ cells had tall microvilli and single cilia forming the brush border (Fig. 4A). COM crystals were found abundantly attached to their surface (Fig. 4A). The surface of MDCK-II cells was covered with microvilli that were less tall and with some cilia that seemed to have affinity for the crystals (Fig. 4B). The surface of MDCK-I cells was characterized by the presence of many small and stubby microvilli, apparently without an affinity for COM crystals (Fig. 4C).

DISCUSSION

Previously, we reported that the affinity of the cell surface of high resistance MDCK cells for COM crystals depended on the developmental stage of the cultures [18, 28]. Crystal binding decreased during the growth of unpolarized precursor cells into confluent and functional monolayers. The level of crystal binding increased during the repair of wounds made in intact monolayers, and decreased after the wounds were healed [18]. During this process, crystals were found to adhere preferentially to migrating cells at the border of the wound. From these observations, it was speculated that the protection against crystal adherence acquired by intact monolayers might reflect a functional property of cells with characteristics of the renal collecting duct. In this study, this idea was further investigated using cell lines with characteristics from different regions of the nephron. The results show that the level of crystal binding to high-resistance MDCK-I and low-resistance MDCK-II cells decreased during their growth to confluency. In contrast, LLC-PK$_1$ cells were unable to avoid crystal adherence, irrespective of their stage of development. To our knowledge there is one other report in which the binding of COM crystals to epithelial cells was studied during their growth to confluency. These studies showed that the level of crystal binding decreased to confluent monolayers of rat inner medullary collecting duct cells, but not to MDCK cells [29]. Although this seems to be in contrast with our results, it should be mentioned that in the latter study, MDCK cells did not form monolayers, but continued to proliferate and piled up into multilayers. We also found that crystals preferentially adhered to stacked MDCK cells at sites where wound borders contacted [18]. Nevertheless, the results obtained with inner medullary collecting duct cells are largely comparable to those obtained in this study with MDCK cells cultured on solid substrates (Fig. 2) and support the idea that late segments of the nephron are protected from crystal binding.

It is often assumed that LLC-PK$_1$ cells originate from the renal proximal tubule [30] and MDCK cells from the renal collecting duct [31–34]. The observation that the proximal tubule brush border marker enzymes γ-GT and AP were abundantly expressed in LLC-PK$_1$ but virtually absent in MDCK-I cells is in agreement with their sup-

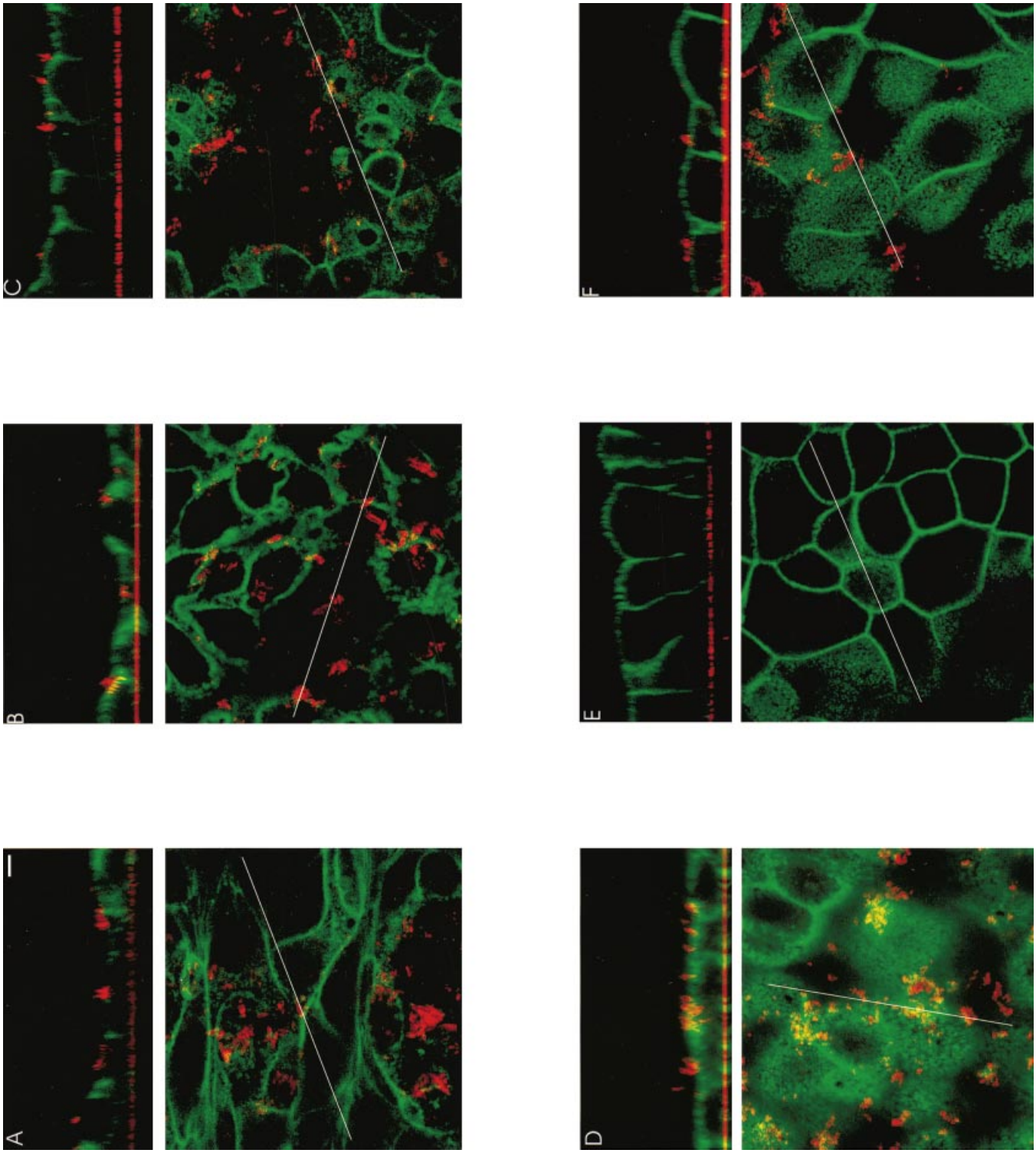


Fig. 3. Confocal microscopy images of the adherence of crystal oxalate monohydrate (COM) crystals to the surface of confluent monolayers of LLC-PK$_1$ (*A*, *B*), MDCK-II (*C*, *D*), and MDCK-I (*E*, *F*) cells, cultured on permeable (A, C, E) and impermeable (B, D, F) growth substrates. Cells are visualized by phalloidin FITC-labeled F-actin (green). The growth substrate, the glass-slide placed on top of the cells, and the COM crystals are shown by light reflection (red). The lines in the horizontal scans (bottom panels) indicate the location of the vertical scans (top panels). These images clearly show that Madin-Darby canine kidney (MDCK) cells cultured on a permeable surface (C, E) are much taller and thus more differentiated compared with cells cultured on a impermeable surface (D, F). This difference in morphological differentiation is less clear in LLC-PK$_1$ cells that have a rather slender appearance cultured on either growth substrate (A, B). Furthermore, these images show the adherence of crystals at the surface of LLC-PK$_1$ (A, B) and MDCK-II (C, D) cells cultured on either growth substrate, whereas crystals are observed at the surface of MDCK-I cells cultured on a solid surface (F), but not to those cultured on a permeable support (E). Bar = 10 μm.

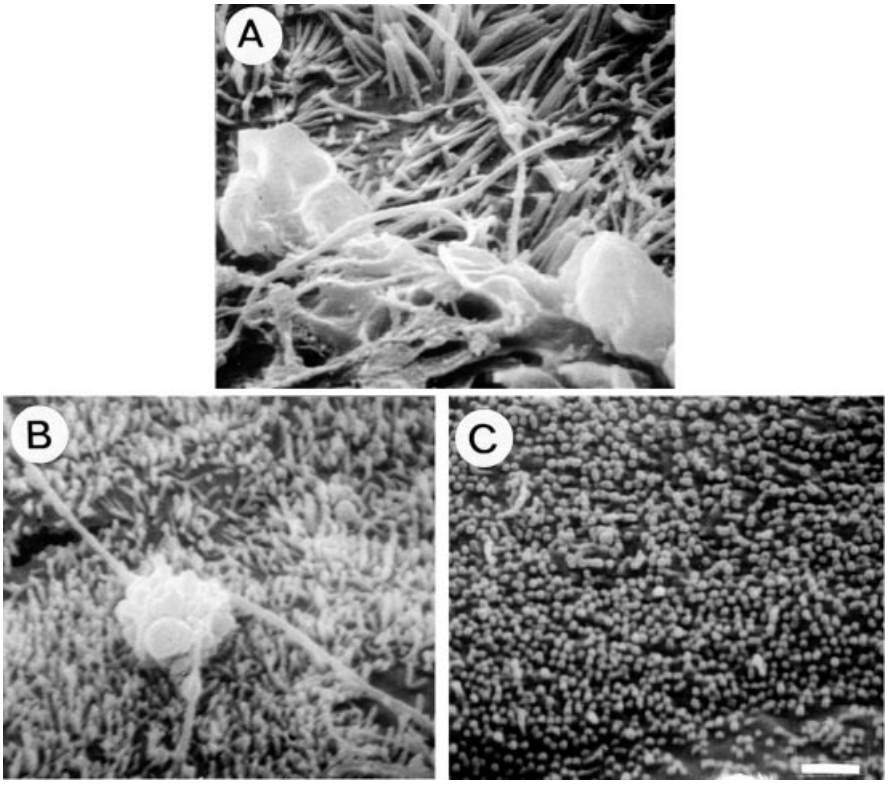
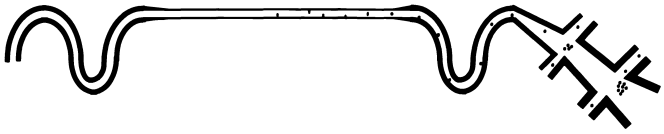


Fig. 4. Scanning electron microscopy images of crystal oxalate monohydrate (COM) crystal binding to the apical surface of functional monolayers formed by LLC-PK$_1$ (*A*), MDCK-II (*B*), or MDCK-I (*C*) cells, cultured on permeable growth substrates. LLC-PK$_1$ cells have tall microvilli and cilia forming the brush border, and COM crystals are found abundantly attached to the cell surface (A). Microvilli of intermediate length and cilia with apparent affinity for COM crystals are seen at the surface of MDCK-II cells (B), whereas the surface of MDCK-I cells is covered by small and stubby microvilli, and no crystals are found attached to its brush border (C). Bar = 1 μm.

posed origin. Substantial γ-GT and AP enzyme activities, however, were also detectable in MDCK-II cells. The original MDCK cell line was established from the whole kidney of a dog, and although it exhibits many morphological and functional similarities with the mammalian cortical collecting duct, it has been recognized that this cell line is not homogeneous. Typical characteristics of intercalated cells of the collecting duct (ICC), such as the binding of peanut agglutinin, as well as the presence of carbonic anhydrase activity were expressed in 30 to 50% of the MDCK cells [31]. Over the years, a number of relatively stable MDCK strains were isolated, including low-resistance strains [25, 31–34] with characteristics of collecting duct α- and β-ICC [33], and high-resistance strains [24, 32–35], with characteristics of collecting duct principal cells [33] and β-ICC [35]. The presence of γ-GT and AP enzyme activities in MDCK-II cells in this study confirms earlier findings [32] and suggests that its brush border shows some resemblance with the proximal tubule. This may explain why the level of crystal binding to MDCK-II cells does not become as low as that observed with MDCK-I cells.

Scanning electron microscopy images revealed an impressive difference in cell surface architecture between the various cell lines. In agreement with the renal proximal tubule, LLC-PK$_1$ cells have abundant, tall microvilli, whereas the villi are much shorter on MDCK-II cells, and villi are distinctly short and stubby on the surface of MDCK-I cells. In general, the apical membrane should protect the cell from the harsh conditions in the outside world. One such condition is the physical contact with calcium oxalate crystals, which can inflict severe damage to tissues [36]. In the bladder, polysaccharide side chains of cell-surface–associated glycoconjugates were proposed as primary defensive barrier to the adhesion of microbes and microliths [19]. The possibility that such a protective cell surface coat also explains the repulsion of crystals by functional MDCK cells warrants further investigation.

The epithelium lining the renal tubules is composed of a single layer of polarized cells. The apical surface faces the lumen, whereas the basolateral side faces the basement membrane. These two plasma membrane domains have different protein and lipid compositions. Each cell in the layer is linked to its neighbors by tight junctions that not only form the permeability barrier between the cells, but also inhibit the lateral diffusion of lipids and proteins between the two plasma membrane domains. We showed that the ability of crystals to become associated with the cell surface decreased rapidly after the tight junctions were formed [18]. Because the polarity of the cell surface increases sharply after tight junction formation [37], we and others suggested that the establishment of epithelial polarity might protect the cell surface from crystal adherence [38]. In this article, we studied in more detail the possible relationship between the development of the differentiated polarized



	PT	DIH	AIH	DT	CD
Retention by	Adhesion	Adhesion	Adhesion	Adhesion	Size/adhesion
Anti-adhesion	No	?	?	Yes	Yes
Crystals present					
Low oxalate	No	Starting	Yes	Yes	Yes
High oxalate	Yes	Yes	Yes	Yes	Yes

Fig. 5. Site of calcium oxalate crystal formation and crystal retention in the various segments of the nephron, as well as epithelial surfaces that may be protected from crystal adhesion. Abbreviations are: ALH, ascending limb of Henle; CD, collecting duct; DLH, descending limb of Henle; DT, distal tubule; PT, proximal tubule. Low oxalate is <1000 μmol per 24 hours and high oxalate is >1000 μmol per 24 hours.

epithelial cell phenotype and COM crystal binding. It was demonstrated that ouabain, which binds specifically to an extracellular domain of the α subunit of active Na^+,K^+-ATPase [39], predominantly binds at the basolateral membrane of confluent filter-grown monolayers formed by MDCK-I, MDCK-II, as well as by LLC-PK_1 cells, indicating that all three cell types formed highly polarized monolayers. Because the degree of epithelial differentiation and polarization also depends on the surface on which the cells are grown [40], we also studied cell morphology and crystal binding to cells cultured on different growth supports. These studies showed that compared with cells grown on a solid substrate, MDCK cells grown on permeable surfaces not only achieved a higher degree of morphological differentiation (Fig. 3), but also obtained higher levels of protection (Fig. 2). These results further support the idea that the ability of crystals to adhere to the MDCK cell surface depends on their stage of differentiation and polarization. Because MDCK-I cells obtained a higher level of polarization than MDCK-II cells (Fig. 1), this could explain the differences in susceptibility to crystal binding between these two strains. On the other hand, polarized monolayers formed by LLC-PK_1 cells appeared to be unable to avoid crystal binding, indicating that epithelial polarization certainly is not a general mechanism to protect the cell surface from crystal binding. It should be emphasized, however, that differentiated epithelial cell phenotypes express only those characteristics that are functionally indispensable and that protection from crystal binding could be expressed only in those segments of the nephron in which such protection is required. From the literature on micropuncture and transport studies, a reasonable impression can be derived on how factors relevant to calcium-salt precipitation change throughout the nephron. Based on these data, several groups have studied the question of whether crystallization can begin inside the nephron and, if so, in which segments it will most likely start [3–8]. It appears that under normal intratubular conditions, calcium-salt crystallization can occur within the time that tubular fluid normally spends in the nephron [4, 6]. It starts with a calcium-phosphate phase under conditions found in the thin limbs. This crystallization phase then (partly) dissolves when collecting duct conditions are approached, thereby inducing the formation of calcium oxalates [4, 6–8]. The relevance of this heterogeneous nucleation is especially clear considering that without these dissolving particles under exactly the same conditions, calcium oxalate formation does not occur within the passage time of fluid through these segments [6]. In the distal tubules and collecting ducts, the crystals may increase in size by crystal growth, but never approach sizes in the range of the tubule diameter [9, 41]. In the collecting ducts, where crystals emerging from numerous nephrons meet, particle size may increase dramatically by crystal aggregation [9]. These data indicate that initially somewhere in the loop of Henle, the presence of crystals will be a normal event. Retention of these particles can take place only by adhesion to the tubular epithelium. In the collecting ducts, particles can be retained by adhesion and because of formation of aggregates that are too large to pass freely. This being so, it is reasonable to expect that in the collecting ducts, additional protection can be expected from controlling the aggregation process [10, 11]. Here, we found that indeed cells with distal/collecting duct characteristics have the ability to prevent crystal adhesion. In contrast, in the proximal tubule, crystallization will not take place unless the oxalate concentrations reach the extreme levels as found in, for example, hereditary hyperoxaluria, ethylene glycol intoxication, or animal models of hyperoxaluria [4, 6]. This puts our current findings that cells of proximal tubule origin cannot prevent crystal adherence into a functional perspective. It also adds a dimension to the risk posed by high oxalate levels. Namely, crystals are formed at sites that are not prepared to avoid their adherence (Fig. 5).

Taken together, the results from this study show that the binding of COM crystals is reduced to functional and polarized monolayers formed by MDCK cells, but not to those formed by LLC-PK_1 cells. MDCK cells cultured on permeable supports obtain a higher degree of

morphological differentiation and are more protected from crystal binding than cells cultured on impermeable growth substrates. These results further support the idea that the anti-adhesion properties acquired by differentiated MDCK cells reflect a functional characteristic of its *in situ* equivalent, the renal collecting duct.

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