Loss of albumin and megalin binding to renal cubilin in rats results in albuminuria after total body irradiation

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Yammani, Raghunatha R., Mukut Sharma, Shakuntla Seetharam, John E. Moulder, Nancy M. Dahms, and Bellur Seetharam. Loss of albumin and megalin binding to renal cubilin in rats results in albuminuria after total body irradiation. Am J Physiol Regulatory Integrative Comp Physiol 283: R339–R346, 2002. First published April 4, 2002; 10.1152/ajpregu.00752.2001.—The role of the renal apical brush-border membrane (BBM) endocytic receptors cubilin and megalin in the onset of albuminuria in rats exposed to a single dose of total body irradiation (TBI) has been investigated. Albuminuria was evident as immunoblot (IB) analysis of the urine samples from TBI rats revealed excretion of large amounts of albumin. IB analysis of the BBM proteins did not reveal any significant changes in cubilin or megalin levels, but 125I-albumin binding to BBM from TBI rats declined by 80% with a fivefold decrease (from 0.5 to 2.5 μM) in the affinity for albumin. IB analysis of cubilin from the BBM demonstrated a 75% loss when purified using albumin, but not intrinsic factor (IF)-cobalamin (Cbl) ligand affinity chromatography. Immunoprecipitation (IP) of Triton X-100 extract of the BBM with antiserum to cubilin followed by IB of the immune complex with an antiserum to megalin revealed a 75% loss of association between megalin and cubilin. IP studies with antiserum to cubilin or megalin and IB with antiserum to the cation-independent mannose 6-phosphate/insulin-like growth factor II-receptor (CIMPR) revealed that CIMPR interacted with both cubilin and megalin. In addition, TBI did not disrupt the association of CIMPR with either cubilin or megalin in BBM. These results suggest that albuminuria noted in TBI rats is due to selective loss of albumin and megalin, but not CIMPR or IF-Cbl binding by cubilin. Furthermore, these results also suggest that albumin and IF-Cbl binding to cubilin occur at distinct sites and that in the rat renal BBM, CIMPR interacts with both cubilin and megalin.

endocytic receptors; endocytosis

cubilin is a 460-kDa multidomain (24) cell surface glycoprotein receptor that is expressed in the intestine, kidney, and yolk sac (31). In the intestine it plays an important role in the uptake of dietary cobalamin (Cbl; vitamin B12), while in the kidney and yolk sac it is thought to play an important role in the endocytosis of many nutrients and lipoproteins (26). Cubilin binds to a variety of ligands such as albumin (4), intrinsic factor (IF)-Cbl complex and receptor-associated protein (5), apolipoprotein A-1 (apoA-1; Refs. 18, 20), transferrin (21), and myeloma light chains (2). The in vivo role of cubilin in the endocytosis of the three ligands IF-Cbl, albumin, and apoA-1 is best exemplified in a canine model that developed vitamin B12 deficiency (14) due to lack of cubilin expression in the renal and intestinal apical brush-border membrane (BBM) (15). In addition to developing vitamin B12 deficiency, these animals also excreted large amounts of albumin (4) and apoA-1 (20) in their urine, demonstrating that loss of renal apical cubilin resulted in a failure of tubular reabsorption of both apoA-1 and albumin.

Albumin reabsorption by the renal proximal tubular epithelial cells is an important physiological process, which prevents its urinary excretion by allowing its uptake by the proximal tubular epithelial cells. In healthy subjects, albumin filtration and reabsorption are in equilibrium, and disequilibrium of this process results in albuminuria (16). Patients with heavy albuminuria are likely to develop tubulointerstitial inflammation, scarring, and fibrosis and progress to end-stage renal failure, and such a progression may be related to slow injury of the epithelial cells (6). Despite the importance of albumin reabsorption, the role of the large endocytic receptors cubilin and megalin in this process is not fully understood. Although cubilin binds to albumin, its endocytosis has been proposed to depend on interaction of cubilin with megalin, another large endocytic receptor of molecular mass 660 kDa (9). The uptake and endocytosis of albumin by the renal proximal tubular epithelial cells appear to involve both cubilin and megalin (41). However, it is not known whether the expression of both these megareceptors at the apical surface or the interaction between them regulates the amount of albumin that is reabsorbed by the proximal tubular epithelial cells. To address some of these issues, we have used a rat model

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in which albuminuria was induced after a single dose of total body irradiation (TBI). Albuminuria noted in these animals has been suggested to be due to increased glomerulus permeability of albumin (36), but the effect of radiation at the tubular reabsorption stage has not been investigated. In our present study, we have demonstrated that albuminuria noted in these animals is due to selective loss of albumin and megalin binding, but not IF-Cbl binding, by renal apical BBM cubulin. In addition, our study also demonstrates that in these animals, the binding of megalin to albumin or CIMPR or the binding of cubulin to CIMPR is not altered significantly, suggesting that under our experimental conditions and the dose of radiation, the damage to apical membrane cubulin is highly selective.

**MATERIALS AND METHODS**

**Materials.** The following were commercially purchased as indicated: [57Co]Cbl (1.3 μCi/μg) and carrier free Na125I (ICN Radiochemicals, Irvine, CA), rat albumin, protein A, and CNBr Sepharose (Sigma, St. Louis, MO). IF used in these studies was prepared from the rat stomach as described earlier (33). Antiserum to purified rat megalin was raised in New Zealand white rabbits as described earlier (39). Antibodies to rat renal cubulin were prepared as described earlier (34). Antiserum to bovine liver CIMPR was prepared as described earlier (12).

**Animals.** The rats used in the study were 9-wk-old WAG/Rij/MCW males that were bred and housed in Animal Research Center facility at the Medical College of Wisconsin, Milwaukee, WI. These animals were free of Mycoplasma pulmonis, Pseudomonas, and common murine viruses. The protocol used in the study had been reviewed and approved by the Animal Care Committee and the Biohazard Committee of the Medical College of Wisconsin.

**Irradiation.** Animals were given TBI with orthovoltage X-rays. The total radiation given in a single dose was 9.5 Gy. Unanesthetized animals were immobilized in a specially constructed Plexiglas jig for irradiation. Animals received a bone marrow transplant immediately after the end of the radiation course (27). Control rats were sham irradiated. Individual irradiated and control rats were kept in metabolic cages designated to collect urine samples. Urine samples were collected for a period of 24 h, and the urine collected was divided into small aliquots and frozen at −70°C. The urine samples were thawed out and used immediately. Some rats were killed 9 wk after irradiation, and tissues (kidney and intestine) were removed, chilled in ice-cold saline for 5 min, and homogenized in 10 mM Tris-HCl buffer.

**Membrane preparations.** Total mucosal membrane from the distal half of the rat intestine was prepared as follows. Mucosa (1–2 g) suspended in 10–20 ml 10 mM Tris-HCl, pH 7.4, containing 50 mM mannitol, 140 mM NaCl, 0.1 mM phenylethylsulfonyl fluoride, and 2 mM benzamidine (buffer A) was homogenized in motor-driven Potter-Elvejhem homogenizer using 10–15 up and down strokes. The homogenate was centrifuged at 100,000 g for 30 min, and the pellet fraction was resuspended and homogenized in buffer A and used as total membranes. Apical BBM from rat kidney was prepared by the Ca2+ precipitation method as described earlier (35). The yield of the apical BBM marker γ-glutamyl transpeptidase was ~17% with 15-fold enrichment. Contamination of the apical BBM by other organelles was between 1 and 2% as determined by specific marker enzyme assays: β-glucuronidase (13) for lysosomes, Na+-K+-ATPase (3) for basolateral membranes, and NADH oxidase (17) for microsomal and outer mitochondrial membranes.

**Iodination of rat albumin and protein A.** Fifty micrograms of rat serum albumin or protein A was iodinated with 0.5 mCi of Na125I and IODO-GEN as recommended by the manufacturer. The iodinated rat albumin was separated on a Sephadex column in 10 mM Tris-HCl buffer, pH 7.4, containing 140 mM NaCl. The recovery was estimated to be ~80%, and the specific activity of both albumin and protein A was 2.5–3 × 106 disintegrations·min−1·μg protein−1.

**Ligand binding activity.** Cubulin activity in the kidney BBM was measured by its ability to bind IF-[57Co]Cbl complex as described earlier (32). Briefly, rat IF-[57Co]Cbl (0.3–1.5 pmol) was incubated with 50 μg of rat kidney BBM protein in the presence of 10 mM Tris-HCl buffer, pH 7.4, containing either 5 mM CaCl2 or 5 mM EDTA. The Ca2+ specific binding of the ligand was calculated as before (32). 125I-albumin (0.75–40 pmol) binding was carried out by rapid filtration method (1). Briefly, 100 μg of rat kidney BBM protein in 10 mM Tris-HCl, pH 7.4, containing 140 mM NaCl were preincubated at 37°C for ~10 min in the presence and absence of 25-fold molar excess of nonradioactive rat serum albumin. Binding of 125I-labeled rat albumin was determined after incubation at 37°C for 1 h. The binding was terminated by the addition of ice-cold 10 mM Tris-HCl, pH 7.4, containing 140 mM NaCl. The contents of the binding mixture were rapidly filtered through 0.45-μm cellulose filters. The tubes were rinsed with 3 ml of the same cold stop reaction and filtered and counted for filter-bound radioactivity. Specific binding was calculated by subtracting nonspecific 125I-labeled rat albumin binding noted in the presence of cold rat serum albumin from total 125I-labeled rat albumin bound to BBM in the absence of nonradioactive rat serum albumin. The association constant $K_a$ was determined by the double reciprocal plot according to Hooper et al. (19).

**Preparation of ligand affinity matrix and ligand affinity chromatography.** Rat serum albumin was coupled to CNBr-activated Sepharose, and rat gastric IF was coupled to Cbl-Sepharose. One milliliter of a 1:1 suspension in 10 mM Tris-HCl buffer, pH 7.4, of the Sepharose-linked ligands (albumin or IF-Cbl) was capable of binding to at least 500–700 ng of purified renal cubulin. One hundred micromolars of rat kidney BBM protein was solubilized in 1 ml of buffer (10 mM Tris-HCl, pH 7.4, of the Sepharose-linked ligands (albumin or IF-Cbl) and used as total membranes. Apical BBM from rat kidney was prepared by the Ca2+ precipitation method as described earlier.
The immunoblots were quantified using AMBIS-radioimaging system, and the intensity of the immunoreacting bands was translated into arbitrary units. The linearity of the band intensity was confirmed with immunoblots generated using pure rat renal cubilin or megalin (200–2,000 ng). The immunoblots are representative data from three separate blotting experiments using membranes isolated from four or five animals in each group.

Association of endocytic receptors in the renal BBM. Association in the BBM of endocytic receptors was carried out essentially by immunoprecipitation with one antiserum, followed by SDS-PAGE analysis of immunopellet and finally immunoblot with antiserum to a different receptor. Isolated apical renal BBM (100 μg protein) from control and TBI rats was solubilized in 1 mL of buffer (10 mM Tris-HCl, pH 7.4, containing 140 mM NaCl) containing Triton X-100 (1%). The Triton X-100-solubilized fraction was subjected to immunoprecipitation with undiluted (5 μl) cubilin or megalin antiserum and 50 μl of a 1:1 suspension of protein A coupled to Sepharose. The immunopellet was boiled with SDS sample buffer to release the proteins bound, and the proteins were then separated on nonreducing SDS-PAGE. The separated proteins were then transferred overnight at 4°C onto Immobilon-P-membrane at constant voltage of 30 V. The membranes were then probed with diluted (1:5,000) antiserum to rat megalin or bovine CIMPR and 125I-protein A. In the case of intestine, because of very low levels of cubilin and megalin expression, total membrane cubilin-megalin association was determined by immunoprecipitation of Triton X-100 extracts of membrane bound to IF-[57Co]Cbl with antiserum to cubilin and megalin as described earlier (39).

RESULTS

Albumin in the urine of irradiated rats. To confirm the presence of albuminuria in the TBI rats, SDS-PAGE analysis of urine from control and TBI rats was performed (Fig. 1A). The protein pattern on SDS-PAGE revealed a strong single protein band of molecular mass 66 kDa in urine of two separate TBI rats (lanes 3 and 4). Low levels of this protein band could also be detected in the urine of two normal rats (lanes 1 and 2). Immunoblot analysis (Fig. 1B) with rat albumin antiserum confirmed that the 66-kDa band was indeed albumin (lane 2), and this band once again was also detected at low levels in the urine from control rats (lane 1). These results demonstrated the presence of strong albuminuria in the TBI rats.

Because both cubilin and megalin are large-sized albumin binding endocytic receptors, they could be the targets for radiation-induced damage that could result in albuminuria due to decreased albumin binding and endocytosis. To test this hypothesis, we first determined the ability of apical BBM to bind albumin, which will be a measure of its binding to both cubilin and megalin. In addition, we also determined the binding of IF-Cbl to the BBM as this ligand is bound only by cubilin, but not megalin.

Kinetics of 125I-rat serum albumin and IF-[57Co]Cbl binding to renal apical BBM from control and TBI rats. Ligand binding studies (Table 1) revealed that albumin binding to the apical BBM isolated from TBI rats declined by 80% from 50 to 10 pmol/mg protein. The association constant $K_a$ for BBM binding of albumin increased to 2.5 μM in TBI rats from ~0.5 μM in control rats (Fig. 2). Unlike changes in albumin binding, IF-Cbl binding to the apical BBM was the same (3.0–3.3 pmol/mg protein) in both control and TBI rats, and there was no change in the affinity for IF-Cbl (data not shown). These initial kinetic studies indicated that

Table 1. IF-[57Co]Cbl and 125I-RSA binding to rat renal BBM

<table>
<thead>
<tr>
<th>Animal</th>
<th>IF-[57Co]Cbl, pmol/mg protein</th>
<th>125I-RSA, pmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.0 ± 0.20</td>
<td>50 ± 3.0</td>
</tr>
<tr>
<td>TBI</td>
<td>3.3 ± 0.11</td>
<td>10 ± 0.7</td>
</tr>
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Values obtained represent means ± SD of triplicate assays performed using brush-border membrane (BBM) isolated from control and TBI rats. The ligand bound/mg protein was calculated from the double-reciprocal plot obtained using 50–100 μg of BBM protein and increasing concentrations of intrinsic factor-[57Co]cobalamin (IF-[57Co]Cbl; 0.5–2 pmol) and 125I-rat serum albumin (125I-RSA; 0.75–40 pmol). Other details of the binding assay are provided in MATERIALS AND METHODS.
either megalin that binds to albumin or cubilin that binds to both albumin and IF-Cbl may be affected in the renal BBM of TBI rats. To explore these possibilities, the total amount of cubilin and megalin present in the BBM and the amount of these proteins that actually bind to albumin and IF-Cbl were determined.

**Immunoblot analysis of renal BBM cubilin and megalin.** Immunoblot of renal BBM proteins separated on SDS-PAGE with monospecific antiserum to rat renal cubilin and megalin (Fig. 3) revealed no significant changes in the BBM megalin and cubilin protein levels in the TBI (lanes 2 and 4) and control (lanes 1 and 3) rats. Immunoblot with megalin antiserum did reveal some lower-sized proteins, and these could be degraded products of megalin. These data coupled with ligand binding data (Table 1) indicated strongly that TBI might selectively inactivate albumin binding to these two proteins. To examine this possibility, Triton X-100 extracts of the BBM were used to purify cubilin by both albumin and IF-Cbl affinity chromatography and megalin by albumin affinity chromatography.

**Immunoblot analysis of cubilin purified from affinity chromatography** (Fig. 4) using rat serum albumin (Fig. 4A) as a ligand revealed nearly 75% less cubilin present in the Triton X-100 extracts of BBM from TBI rats (lane 2) compared with cubilin purified from normal rats (lane 1). Since albumin binding is also a property of megalin, the same detergent extracts were used to purify megalin using albumin affinity chromatography. Immunoblot analysis (Fig. 4A) revealed that megalin levels purified were the same in both control (lane 3) and TBI (lane 4) rats. Similar immunoblot analysis of cubilin purified using IF-Cbl as the affinity ligand revealed (Fig. 4B) identical levels of cubilin in Triton X-100 extracts of BBM from control (lane 2) and TBI rats (lane 1). In addition to the 460-kDa cubilin band, another band of higher molecular mass was also detected in the immunoblot using the cubilin purified from the BBM of both control and TBI rats using IF-Cbl affinity chromatography. This higher molecular mass protein band was absent in the purified cubilin fraction purified from the BBM of both control and TBI rats using albumin affinity chromatography.

**Quantitative analysis of the image density of immunoblots** (Fig. 4, A and B) revealed a decline of cubilin protein levels in the BBM extracts of TBI rats by five- to sixfold relative to their levels in control rat BBM extracts when these fractions were subjected to rat serum albumin-Sepharose affinity chromatography (Fig. 5). However, similar quantitation of the cubilin protein bands purified on IF-Cbl-Sepharose column did not reveal any significant changes in cubilin protein levels. These results indicated that in rat renal BBM of TBI rats, the albumin binding region of cubilin is...
selectively altered. Although decreased albumin binding alone can explain the onset of albuminuria in TBI rats, inhibition of endocytosis of residual albumin bound to cubilin may also be inhibited because of TBI, which can damage the formation of the cubilin-megalin complex.

**Disruption of cubilin-megalin but not CIMPR-megalin association in the renal BBM of TBI rats.** To determine whether the amount of cubilin associated with megalin is altered due to irradiation, Triton X-100 extracts of the apical BBM were subjected to immunoprecipitation with antiserum to cubilin, and the immune complex was then subjected to immunoblot with antisera to megalin. The data (Fig. 6A) clearly show that cubilin-megalin interaction is disrupted in TBI rat renal BBM (lane 2), and the loss of this interaction was ~75% compared with control rat BBM (lane 1). Because both cubilin and megalin are also expressed, but at low levels, in the distal regions of the rat intestine, we wanted to test whether in TBI rats intestinal BBM cubilin-megalin association is also disrupted. Interestingly, we could not detect any significant changes in the association between cubilin and megalin in the intestinal BBM as nearly 10% of the cubilin-bound IF-[^57]CoCbl could be immunoprecipitated in the Triton X-100 extracts of BBM from both control and TBI rats (data not shown). This observation suggested that under our experimental conditions, TBI damage involving cubilin and megalin appears to be restricted to renal BBM.

To further define the specific nature of the disruption of cubilin-megalin interaction in the renal BBM of TBI rats, we also tested whether TBI affected the association of megalin with any other BBM-resident large-sized endocytic receptor. We chose CIMPR, an endocytic receptor of molecular mass 300 kDa, which has been demonstrated, like megalin, to be localized to the rat renal apical BBM and in clathrin-coated pits (11). Immunoprecipitation with antisera to either cubilin or megalin followed by immunoblot with CIMPR antiserum clearly indicated (Fig. 6B) that CIMPR is indeed associated with cubilin (lanes 1 and 2) and megalin (lanes 3 and 4) and that TBI had no effect on the association of CIMPR with either cubilin or megalin.

**DISCUSSION**

In the current study, we have investigated the potential role of cubilin and megalin in the pathogenesis of albuminuria noted in TBI rats. It is estimated that albumin reabsorption capacity in rats (30) is about 10–135 mg/l, and in humans nearl y7go falbumin is reabsorbed within a 24-h period (7). Defective reabsorption of albumin by the proximal tubular epithelial cells results in proteinuria and is often preceded by apical BBM damage. Albuminuria is known to occur in rats during aging (8), with polycystic kidney disease (29), in early-stage diabetes (37), and in rats that are exposed to TBI (36). Although increased glomerulus permeability of albumin has been noted in many causes of albuminuria, the role of the large albumin receptors megalin and cubilin expressed in the tubular epithelial apical BBM in the reabsorption of albumin and pathogenesis of albuminuria is not well understood.

In our rat model, a single dose of TBI resulted in albuminuria as evidenced by the urinary excretion of albumin (Fig. 1). To understand the cause of albuminuria in these rats, we focused our studies on the two albumin binding receptors in the BBM, cubilin and megalin. Cubilin and megalin, because of their large molecular masses of 460 and 660 kDa, respectively, could be the likely targets for radiation-induced damage and loss from the BBM. However, immunoblot studies (Fig. 3) did not reveal any significant changes in their BBM protein levels in control and TBI rats.

The observation that megalin protein levels (Fig. 3) and its ability to bind albumin (Fig. 4A, lanes 3 and 4) were not altered in TBI rats is interesting and suggested strongly that albumin binding to megalin by itself may not be important for its tubular reabsorption. Earlier studies in megalin knockout mice have indicated no significant increase in albuminuria (22), and only very mild albuminuria was present in the human homolog of murine megalin deficiency (28). In addition, lack of evidence for the direct role for megalin in the tubular reabsorption of albumin has also been shown in rats with early-stage diabetes (37). Albuminuria in these animals has been proposed to be due to causes such as increased lipid peroxidation and decreased endocytosis as megalin levels in these rats decreased by only 10% (37). Despite these studies that have suggested lack of a direct role of megalin in
albumin endocytosis, there is evidence from a later study (4) from megalin knockout mice that albumin excretion in these animals increased threefold. Taken together, these studies have suggested that cell surface expression of megalin may play an indirect role in the endocytosis of albumin, and our data of disruption of megalin-cubilin interaction in causing albuminuria in TBI rats support such an indirect role for megalin.

In contrast to megalin, our studies indicate that cubilin plays a more important role in albumin reabsorption. The following line of evidence supports such a conclusion. First, albumin binding to BBM was reduced by nearly 80% (Table 1) without loss of BBM cubilin levels (Fig. 3) in TBI rat kidney BBM. Second, albumin binding capacity of BBM from TBI rats is reduced (Fig. 2) by fivefold. Albumin binding kinetic values \( K_d \) and \( B_{max} \) (50 pmol/mg protein) reported in this study using control rat renal BBM are very close to \( K_d \) and \( B_{max} \) values (0.43 \( \mu M \) and 40 pmol/mg protein, respectively) reported in another study (1). In addition, the \( K_d \) for albumin binding to rat renal purified cubilin has been reported to be 0.63 \( \mu M \) (4). Finally, the amount of cubilin recovered from the BBM extracts of TBI rats by albumin affinity chromatography was much lower (75%) than that recovered using extracts obtained from the BBM of control rats (Fig. 4A, lanes 1 and 2). However, when fractionated on IF-Cbl affinity chromatography, cubilin levels recovered from both control and TBI rat BBM extracts were similar (Fig. 4B, lanes 1 and 2). This last observation is interesting as it provides two important insights into ligand binding properties of cubilin.

Earlier studies (41) based on differential inhibition by receptor-associated protein of albumin and IF-Cbl binding by cubilin had suggested that IF-Cbl and albumin binding sites could be distinguished. This suggestion was further supported by our earlier studies (40), which were based on differential inhibition of these two ligands binding to cubilin by antiserum to epidermal growth factor. The present study has confirmed these earlier suggestions by providing more direct evidence that albumin and IF-Cbl ligand binding sites of cubilin are different. Two earlier studies have shown that a small percentage of rat renal BBM cubilin also exists as a trimer (39) and that purified cubilin in vitro forms noncovalent trimers connected by NH2-terminal coiled-coil helix (23). Thus the cubilin band of molecular mass >460 kDa noted when purified from IF-Cbl affinity chromatography (Fig. 4B) could represent functional cubilin trimer that is able to bind only IF-Cbl, but not albumin, as this band was absent even in control rat BBM when cubilin was purified by albumin affinity chromatography (Fig. 4A, lanes 1 and 2). The distinct nature of albumin and IF-Cbl binding sites of cubilin may help explain why some patients with Grasbeck-Imerslund syndrome who develop Cbl deficiency due to inherited intestinal malabsorption of Cbl do not develop proteinuria (10, 26). It is possible that different mutations of the cubilin molecule may exist that affect uptake of IF-Cbl, or albumin, or both.

Another interesting aspect of our study is the significant (75%) loss noted in the amount of cubilin associated with megalin (Fig. 6A, lanes 1 and 2) after a single dose of TBI. Loss of cubilin-megalin association appears to be specific for the kidney BBM as it was not detected in the ileal BBM (data not shown). Furthermore, in the kidney, decreased association of megalin occurred with cubilin, but not with CIMPR (Fig. 6B). The specific loss of cubilin-megalin interaction in the renal BBM raises the question whether the loss of albumin binding by renal cubilin is due to loss of its association with megalin. However, this is highly unlikely because cubilin fragments (the 113-residue NH2-terminus or CUB domains 6–8) when expressed in vitro bound albumin in the absence of megalin (40). Thus, while albumin binding by cubilin is independent of its interaction with megalin, their association in the apical BBM appears to play a role in the endocytosis of
albumin and other ligands that bind to cubilin (25). We have shown earlier (39) that the NH2-terminal region of cubilin binds to megalin in a Ca2+-dependent manner. It is not known whether such interaction with megalin involves any other regions of cubilin. Further studies are needed to address this issue.

Because of the lack of a transmembrane domain in cubilin (24), the endocytosis of many of its ligands is thought to be mediated by its interaction with megalin (25). It is even suggested that synthesis of megalin (18) is to some extent (20%) responsible for the cell surface expression of cubilin. These earlier studies have proposed that megalin acts as a coreceptor active in the endocytosis and intracellular trafficking of cubilin. The current data support such a role of megalin by providing evidence for the existence of cubilin-megalin interactions in the native renal BBM and its importance in the endocytosis of albumin.

In addition to cubilin-megalin interactions in the native BBM, our results also suggest that CIMPR, another endocytic receptor, also interacts with both BBM megalin and cubilin (Fig. 6). At present, the physiological significance, if any, of CIMPR interactions with either cubilin or megalin in the renal BBM is not known. In this context, it is interesting to note that in an adult rat male reproductive system, cubilin endocytosis is mediated via its interaction with low-density lipoprotein receptor-related protein-2 (38). Thus a distinct possibility exists that CIMPR could under some conditions function as a coreceptor for renal cubilin. However, this possibility is unlikely as far as endocytosis of cubilin-bound albumin is concerned because reduced albumin reabsorption was noted in megalin knockout mice (4). Despite our current lack of understanding of the physiological importance of interactions of CIMPR with cubilin or megalin, to the best of our knowledge, this study is the first to demonstrate the association of cubilin and megalin with CIMPR in the rat renal BBM. On the basis of this finding, we propose a model (Fig. 7) to illustrate the functional topography of these three endocytic receptors, two of which, megalin and CIMPR, contain both a transmembrane domain and a COOH-terminal cytoplasmic tail.

In summary, these studies have shown that albumin and megalin binding by renal apical BBM cubilin play an important role in the endocytosis of filtered albumin and that megalin plays only an indirect role in this process. Further studies are needed to address the issues regarding 1) the effect on cubilin interactions with albumin and megalin in other causes of albuminuria and 2) the structural basis for the albumin and megalin binding by cubilin in health and disease.

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REFERENCES


