Myeloma light chains are ligands for cubilin (gp280)

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Myeloma light chains are ligands for cubilin (gp280). Am. J. Physiol. 275 (Renal Physiol. 44): F246–F254, 1998.—Although myeloma light chains are known to undergo receptor-mediated endocytosis in the kidney, the molecular identity of the receptor has not been characterized. We examined the interaction between cubilin (gp280) and four species of light chains isolated from the urine of patients with multiple myeloma. Four lines of evidence identify cubilin, a giant glycoprotein receptor, which is restricted in distribution to endocytic scavenger pathways and which has potent effects on endosomal trafficking, as a potentially physiologically relevant binding site for light chains: 1) light chains coeluted during immunoaffinity purification of cubilin; 2) polyclonal antiserum to cubilin but not control sera, displaced human light chains to cubilin but also a role for cubilin in light chain endocytosis and cell trafficking of light chains. We therefore examined light chain interactions with cubilin. Our data show not only structural binding of light chains to cubilin but also a role for cubilin in light chain endocytosis. Furthermore, a potent effect of light chains on endosomal fusion reconstituted in vitro suggests that excess light chain may be disruptive to cell trafficking.

IMMUNOGLOBULIN LIGHT CHAINS are filtered at the glomerulus and endocytosed in the proximal tubule (2, 3). In overproduction states, such as multiple myeloma, light chains, also known as Bence-Jones proteins, may produce nephrotoxicity. We have shown previously that free κ- and λ-light chain isotopes bind to a single class of renal proximal tubular receptors that facilitate internalization and degradation (3). To date, however, the receptor(s) that mediates endocytosis of light chains in the proximal tubule has not been characterized. Identification of renal binding proteins for myeloma light chains not only leads to a better understanding of normal light chain metabolism in the kidney but should also permit development of protective agents for light chain nephrotoxicity.

METHODS AND MATERIALS

Animals, reagents, and antibodies. Male Sprague-Dawley rats (200–250 g) were from Sasco, Omaha, NE, and all other reagents were from Sigma Chemical (St. Louis, MO) unless otherwise stated. Polyclonal antibodies against cubilin and megalin were raised against proteins purified by immunoaffinity chromatography using previously reported monoclonal antibodies coupled to Sepharose 4B (1, 24, 25). These antibodies are monospecific by immunoblotting on whole brush border preparations and by immunoprecipitation of biosynthetically labeled yolk sac epithelial cells (24, 25), and they bind multiple domains of cubilin and megalin (8, 11, 25). Both are IgG antibodies, used at similar protein concentrations and in identical titers. Control antisera included normal rabbit serum and polyclonal rabbit antiserum to the neurokinin-1 substance P, NK1, receptor (a gift from Professor Jean-Yves Courard, Gif-Sur-Yvette, France).

Preparation of light chains. Four species of light chains, two κ and two λ, were isolated and purified from the urine of four different patients with myeloma, as previously described (2, 3). The purity and the immunologic identity of light chains were confirmed by SDS-PAGE and Western blotting. One of
the \( \lambda \)-light chains and the \( \kappa \)-light chain used here were the same light chains used in a previous report from our laboratory demonstrating receptor-mediated endocytosis by radioisotope techniques (3). Competition experiments were initially conducted using radiolabeled \( \lambda \)-light chain, iodinated by the Iodobead method as previously reported from our laboratories (2, 3). We later switched to competition experiments with FITC-conjugated light chains. FITC conjugation was performed using FluorTag FITC Conjugation Kit (Sigma Immunochemicals, St. Louis, MO). Using this technique, we usually obtained FITC-conjugated light chain protein with a fluorescein-to-protein ratio of \( \sim 0.6 \) to 1.0. Conjugated light chain migrated at nearly the same molecular weight region as unlabeled light chain in SDS-PAGE.

Preparation of renal brush-border membrane vesicles and cortical intermicrovillar clefts. Rat renal cortical brush-border membrane vesicles were isolated by magnesium precipitation and differential centrifugation technique as described previously (2, 5, 12). Rat renal cortical intermicrovillar clefts were prepared from cortical homogenates of kidneys harvested from anesthetized rats, with differential Percoll gradient centrifugation and magnesium precipitation (10, 11). We have also shown that the intermicrovillar clefts form vesicles oriented "cytosolic facade out" in vitro during homogenization and can capture internally components added to the homogenization buffer (11).

Preparation of cubilin. Intermicrovillar clefts prepared from renal cortices were biotinylated on the cytosolic facade using NHS-biotin (19). Cubilin and the associated proteins were purified by immunofinity chromatography in which MAb 75 was coupled to CNBr-activated Sepharose 4B (Pharmacia, Saint Quentin en Yvelines, France) as previously described (24, 25). Protease inhibitors were added at all steps.

Competition between light chains and anti-cubilin and anti-megalin antisera for rat renal brush-border membrane binding. Binding of either \( \lambda \)-labeled or FITC-conjugated light chains was investigated in the presence of up to 100,000-fold serial dilutions of anti-cubilin antibodies (1, 11, 24, 25). Equal dilutions of bovine serum albumin served as controls. With the radiolabeled light chain, binding was assayed in a gamma counter as previously described (2, 3). Binding of FITC-conjugated light chains was assayed using small particle techniques on a Becton-Dickinson FACStar flow cytometer with a Consort 30 computer and WinMidi software (7, 11, 24, 25). The analog-to-digital conversion of fluorescence measurements on each particle passes through a logarithmic amplifier such that fluorescence is expressed on a log scale.

Surface plasmon resonance analysis of light chain/cubilin interaction. Direct binding analysis utilized surface plasmon resonance by BIAcore technology (5a, 30). In this technique, a prism sits with its flat surface on a gold sheet. The angle of reflection of laser light through the prism depends on the molecular weight of the compound bound to the gold chip. After covalent attachment of light chains or other proteins to the gold surface, ligand binding is detected in real time by monitoring the angle of reflection of the laser light through the prism. Four identical chambers in parallel have increasing concentrations of light chain protein bound covalently, and the same ligand stock is presented to each chamber simultaneously in a binding buffer. This technique allows direct determination of ligand receptor interactions. The \( \kappa \) or \( \lambda \)-light chains were immobilized via free amine groups to the dextran matrix of CM5 sensor chips activated by a 1/1 mixture of N-hydroxysuccinimide and N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide HCl. Unreacted sites were blocked with 1 M ethanolamine, pH 8.5 (5a, 30). The immobilization was conducted at 25°C using 10 mM HEPES, 2 mM CaCl\(_2\), 150 mM NaCl, and 0.005% Nonidet P-40, pH 7.4, as the flow buffer. Electrostatic preconcentration of light chains at various pH values was used as an index of the protein's isoelectric point to optimize covalent binding. Optimal condition was achieved with 10 mM acetate at pH 4.8, which was used in all subsequent experiments. Different densities of \( \kappa \) - or \( \lambda \)-light chains were immobilized to three of the four flow cells; the remaining flow cell was activated and blocked with no light chains immobilized for use as a control surface. Binding experiments were carried out using a BIAcore 2000 instrument (Pharmacia USA, Piscataway, NJ). To control for nonspecific binding to light chains, we passed bovine serum albumin, casein, or \( \beta \)-lactoglobulin over the light chain chip at molar concentrations matching or exceeding levels used in cubilin dose-response experiments (up to 1 mM) and observed no detectable binding. Conversely, to control for nonspecific cubilin binding, we prepared albumin and casein bound chips. There was no detectable cubilin binding to the albumin or casein cross-linked chips.

Identification by two-dimensional gel electrophoresis and microsequencing of proteins associated with immunopurified cubilin. The approach we used involved three steps: 1) biotinylation of intermicrovillar membranes, 2) immunosolution of cubilin, and 3) identification of bound proteins by microsequencing. Two-dimensional electrophoresis was performed according to the method of O’Farrell (20) by Kendrick Labs (Madison, WI). Proteins other than cubilin observed on two-dimensional gels prepared from the eluate of detergent solubilization of intermicrovillar clefts were identified by microsequencing. For this purpose, three gels were run in parallel, and stained with Coomassie D. The two most abundant spots at molecular mass 56 and 24 kDa from each gel were cut out, and the material was pooled. The peptides derived from the eluted proteins by leucine-aminopeptidase (C-leu) digestion were separated by HPLC, and internal peptides were sequenced (6).

Effect of light chains on endosomal fusion. To determine whether light chains had a direct effect on membrane fusion, rat renal cortical intermicrovillar clefts were prepared as previously described (8, 11). Aliquots of microvillar clefts were loaded with 0.8 mg/mL FITC-dextran or 1.6 mg/mL Lissamine rhodamine dextran by addition of the fluorescent dextrans to the homogenization buffer. Some aliquots of these fluorescent dextran-loaded intermicrovillar clefts also had 400 µM unlabeled light chain loaded simultaneously. Fusion of these light chain-loaded endosomes was compared with control endosomes in which albumin replaced light chains. Changes in fluorescence were detected by flow cytometry using a Becton-Dickinson FACStar flow cytometer with a Consort 30 computer and WinMidi software as described above. When endosomes fuse in vitro, emission of fluorescence increases (9, 10). Data are expressed as means ± SE throughout this report. Statistical analysis was performed by analysis of variance, Mann-Whitney U test, and Bonferroni or Scheffé’s post hoc comparison where appropriate.

Culture of rat visceral yolk sac cells and internalization experiments. The yolk sac epithelial cell line (BN/MSV) was derived from yolk sac teratocarcinoma induced by fetectomy and placent injection of mouse sarcoma virus (24). When grown under conventional conditions in modified Eagle’s medium supplemented with 2.5 mM L-glutamine, 10% fetal calf serum, and an antibiotic cocktail (penicillin, streptomycin, and fungizone), the cells form a domed monolayer and express abundant cubilin (24).

Effect of anti-cubilin antisera on endocytosis of light chains. Internalization experiments were conducted by expos-
ing confluent yolk sac cells in 24-cell plates to 50 μM FITC-conjugated light chain. These cells were selected for endocytosis experiments because cubilin expression is ∼100-fold greater than cultured proximal tubule cells (24). Cells were allowed to endocytose FITC-light chain at various intervals for up to 40 min at 37°C with and without polyclonal anticubilin antibody at 1:1,000 dilution (added at time 0). This concentration is selected because it is 10-fold higher than the half-maximal inhibitory concentration of the antibody determined from the brush border binding inhibition experiments. Endocytosis is stopped by washing twice with PBS and removing light chain from medium. Cells are then trypsinized, fixed in 1% formaldehyde, and suspended in PBS, and FITC incorporated into each cell is read in a Becton-Dickinson flow cytometer as described above. Endocytosis curves are generated by plotting fluorescence units against time. Excess unlabeled light tosis curves are generated by plotting fluorescence units corrected for background against time. Excess unlabeled light chain was used to test for specificity, and bovine serum albumin was used as nonspecific protein control.

RESULTS

Four lines of evidence indicate that light chains are ligands for the scavenger pathway receptor cubilin.

Evidence 1. To identify candidate ligands with which cubilin interacts, we subjected a detergent extract of rat renal apical intermicrovillar clefs biotinylated on their cytosolic facade to affinity chromatography. The extract was passed through an immunoaffinity column prepared with polyclonal anti-cubilin antiserum raised against confluent yolk sac cells in 24-cell plates to 50 μM FITC-conjugated light chain. These cells were selected for endocytosis experiments because cubilin expression is ∼100-fold greater than cultured proximal tubule cells (24). Cells were allowed to endocytose FITC-light chain at various intervals for up to 40 min at 37°C with and without polyclonal anticubilin antibody at 1:1,000 dilution (added at time 0). This concentration is selected because it is 10-fold higher than the half-maximal inhibitory concentration of the antibody determined from the brush border binding inhibition experiments. Endocytosis is stopped by washing twice with PBS and removing light chain from medium. Cells are then trypsinized, fixed in 1% formaldehyde, and suspended in PBS, and FITC incorporated into each cell is read in a Becton-Dickinson flow cytometer as described above. Endocytosis curves are generated by plotting fluorescence units corrected for background against time. Excess unlabeled light chain was used to test for specificity, and bovine serum albumin was used as nonspecific protein control.

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Evidence 1. To identify candidate ligands with which cubilin interacts, we subjected a detergent extract of rat renal apical intermicrovillar clefs biotinylated on their cytosolic facade to affinity chromatography. The extract was passed through an immunoaffinity column prepared with polyclonal anti-cubilin antiserum raised against the whole molecule. Western blot analysis of the eluate using the same antibody showed a single band at the region ∼460–540 kDa, consistent with cubilin (Fig. 1A, left lane). There has been some confusion about the molecular weight of cubilin. Cubilin was originally known as gp280 (280-kDa glycoprotein), based on the observation that it was a little smaller than gp330, now cloned and named as megalin. However, electrophoresis gels are notoriously inaccurate in estimating molecular size of large proteins. Indeed, when megalin was cloned (26), it proved to be 600 kDa, and cubilin's size was reestimated at 540 kDa. When physiological and immunologic evidence proved cubilin identical to the intrinsic factor-cobalamin receptor, it was again reestimated at 460 kDa (28). Cloning cubilin demonstrated an amino acid sequence of 400 kDa (19). Deglycosylation of 460-kDa membrane-bound form of cubilin yields a 400-kDa protein matching the molecular mass predicted from the amino acid sequence. Coomassie staining of a parallel gel revealed several additional bands (Fig. 1A, right lane). For further characterization, the proteins eluted from the column were separated by two-dimensional gel electrophoresis and transferred, and the spots were cut out of the gels (Fig. 1B). Pooled material representing the same spot from multiple gels was C-leu digested, and fragments were separated by HPLC and microsequenced (6). Proteins eluted from the column included cubilin (Fig. 1, left at top of gel), a 56-kDa protein identified as the
β-subunit of the H^+-ATPase by the sequence VVDLLAPYA ("#1"), a 24-kDa protein identified as κ-light chains by the sequence (I/S)PQLLYNA ("#2"), and an internal tropomyosin control protein added exogenously to the gel (solid arrow). The 56-kDa protein was biotinylated, suggesting cytosolic residence, and hence was not pursued as a ligand; whereas the 24-kDa protein was not biotinylated, suggesting exofacial residence (Fig. 1, bottom).

Evidence 2. At this point it was still uncertain whether light chains are a ligand for cubilin or were merely eluting from the antibody on the column. Analysis of cubilin binding to κ- and λ-light chains using surface plasma resonance techniques provides direct evidence that cubilin binds light chains. A stock solution of cubilin was diluted serially with flow buffer and passed over the immobilized κ-light chain surfaces for 5 min (50 µl at 10 µl/min, 25°C), followed by monitoring the dissociation phase induced by introduction of cubilin free-flow buffer for 4 min (Fig. 2A). After 4 min, the cubilin bound to the surface had dissociated completely, so it was not necessary to regenerate the surface prior to the next injection. The sensorgrams were corrected for bulk refractive index changes by subtracting the response on the blank flow cell from the other flow cells. Cubilin bound to κ-light chains in a dose-dependent fashion (Fig. 2A).

To further demonstrate the binding specificity of the cubilin to the immobilized κ-light chains, a competition experiment was conducted. A sample of cubilin (100 nM) was incubated with κ-light chains (10 or 490 µM) or λ-light chains prior to mixing the sample over the κ-light chain surface. The binding of cubilin to the immobilized surface was reduced in the presence of κ-light chains in a dose-response fashion (Fig. 2B). Inhibition of cubilin binding to immobilized κ-light chains with 10 µM λ-light chains suggests κ- and λ-light chains share a common binding site on cubilin. This series of experiments was repeated with immobilized λ-light chains, with four different light chains competing (two λ and two κ) with similar results (data not shown). These studies showed cubilin bound λ-light

Fig. 2. Direct binding analysis of cubilin and myeloma light chains by surface plasmon resonance. A: gp280 binding to κ-light chains surface-Fc1 3975 RU; binding of cubilin to immobilized κ-light chains is dose dependent with rapid low-affinity association and dissociation kinetics. B: a solution of inhibition (competition) experiment (gp280 + κ- or λ-light chains) further demonstrates the specificity of binding of cubilin to immobilized κ-light chains. A sample of cubilin (100 nM) was incubated with κ-light chains (10 or 490 µM) or λ-light chains (10 or 490 µM) prior to mixing the sample over the κ-light chain surface. Binding of cubilin to the immobilized surfaces was reduced in the presence of κ- or λ-light chains in a dose-response fashion. Data are representative of experiments with 4 light chains on 3 chips. C: effect of temperature on binding of gp280 (110 nM) to λ-light chains surfaces; binding of cubilin to light chains is temperature dependent. LC, light chains; RU, response units.
chains in a dose-dependent fashion, and binding was interfered with in a dose-response fashion by both free \( \lambda \) and \( \kappa \)-light chains. In these studies, bovine serum albumin neither competed with light chains nor bound to cubilin.

Binding of cubilin to \( \kappa \)-light chains was much greater at 37°C than 25°C (Fig. 2), consistent with known thermal behavior of receptor-ligand interactions (2). Hence, BIACORE surface plasmon resonance analysis allows for direct realtime assay of the binding of myeloma light chains to cubilin, providing direct evidence that cubilin is a renal light chain receptor.

Evidence 3. To determine whether light chains bind to cubilin present in brush-border membranes in its native membrane-bound form, we tested for antibody interference with light chain binding to rat kidney brush-border membrane vesicles, which are known to express cubilin (24). Binding of \( ^{125}I \)-labeled human \( \lambda \)-light chain to rat renal brush-border membrane vesicles is displaced by polyclonal antibodies to cubilin. The half-maximal inhibitory concentration of anti-cubilin antibody was observed at \(-10,000\) dilution (Fig. 3A, solid circles). In contrast, antiserum to megalin, which is known to bind these membranes (18), had no effect on the binding of this light chain (Fig. 3A, open circles), suggesting that this \( \lambda \)-light chain binds exclusively to cubilin. At the maximal inhibitory concentration, the anti-cubilin antiserum displacement of \( \lambda \)-light chain approached 90%, confirming near exclusive binding of this light chain to cubilin. We also observed that binding of human FITC-conjugated \( \kappa \)-light chain to rat renal brush-border membrane vesicles was displaced by polyclonal antibodies to cubilin as assayed by flow cytometry (Fig. 3B). Light chain binding (45.5 \( \pm \) 4.3 arbitrary fluorescent units, \( n = 8 \)) increased compared with unstained membranes (5.1 \( \pm \) 1.2 units, \( n = 8 \), \( P < 0.05 \)) and was displaced by anti-cubilin antibody (30.2 \( \pm \) 1.0 units, \( n = 8 \), \( P < 0.05 \)). There was no effect on light chain binding by normal rabbit serum (42.9 \( \pm \) 1.7 units, \( n = 8 \)) or antiserum to the neurokinin-1/substance P receptor (40.0 \( \pm \) 1.2 units, \( n = 4 \)), an irrelevant antibody which binds these membranes. This provides additional evidence that the competitive effect of cubilin antiserum on the binding of light chain is specific.

Flow cytometry histograms of light chain binding on a vesicle-by-vesicle basis illustrate the effects of cubilin antiserum on rat renal brush border binding of FITC-\( \lambda \)-light chains. Each histogram (Fig. 3B) displays 2,000 vesicles as individual dots, with FITC fluorescence plotted against vesicle size. FITC-light chains bound to most but not all brush borders (Fig. 3B, left). Cubilin antiserum displaced FITC light chain binding (Fig. 3B, right).

Evidence 4. To examine the role of cubilin in light chain endocytosis, yolk sac cells were allowed to endocytose FITC-light chain in the absence and presence of anti-cubilin antiserum. These endocytosis experiments revealed a significant inhibitory effect but not total elimination of endocytosis (Fig. 4). Excess unlabeled light chain and anti-cubilin antibody reduced FITC-light chain endocytosis significantly (\( n = 4 \), \( P < 0.002 \), Mann-Whitney-U test), whereas albumin had no effect (Fig. 4A). Furthermore, a time course study showed that anti-cubilin antiserum inhibited light chain endocytosis significantly at all time intervals studied (Fig. 4B, \( n = 3 \) each time period, \( P < 0.0001 \)). This time course experiment also showed that anti-cubilin antiserum eliminated the saturable pattern of endocytosis with apparent linearization of the uptake curve (Fig. 4B). This observation further supports that cubilin mediates light chain endocytosis in yolk sac cells. Less than complete inhibition of light chain endocytosis in the presence of anti-cubilin antiserum also indicates that, when this pathway is blocked, some light chain endocytosis occurs through alternate pathways, and
that the cubilin-facilitated path is not the exclusive endocytic pathway for light chains.

Evidence for functional role. To test whether myeloma light chains are functionally important in membrane trafficking and fusion events, intermicrivialicle fects were loaded with light chain by adding it to the homogenization buffer (10, 11). Fusion reconstituted in vitro in cuvettes was assayed by energy transfer, and results were normalized per milligram protein (11, 14). Fusion was significantly inhibited in membranes treated directly with light chains (111 ± 89 arbitrary fluorescence units/mg protein) compared with albumin-entrapped controls (1,584 ± 314, n = 8, P < 0.0003 by unpaired t-test, Fig. 5).

**DISCUSSION**

These studies show that cubilin, a giant receptor which participates in the endocytic scavenger pathway of the renal proximal tubule cells, binds and facilitates endocytosis of immunoglobulin light chains isolated from the urine of myeloma patients. The first evidence that cubilin is a light chain receptor came from the analysis of eluates from an affinity column prepared with anti-cubilin antiserum in which cubilin coeluted with κ-light chain (Fig. 1). The κ-light chain was definitively identified by microsequencing after isolation by two-dimensional electrophoresis. Several additional lines of evidence add weight to the hypothesis that cubilin is a light chain receptor. Competition experiments by anti-cubilin antiserum and surface plasmon resonance experiments both showed that all tested light chains bind to cubilin.

Surface plasmon resonance technology allowed direct analysis of the binding of light chains to cubilin. Several characteristics of the observed sensorgrams suggest that light chains bind cubilin specifically. First, cubilin bound to light chains in a temperature- and dose-dependent manner whether κ- or λ-light chain is immobilized. Second, four species of nonimmobilized light chains all interfered with cubilin binding in a dose-dependent manner. Third, the kinetics of binding and displacement were very similar to values reported previously using radioactive membrane binding techniques (2, 3, 5). Last, λ-light chains interfere with κ-light chain binding to cubilin and vice versa. This data revalidates the use of surface plasmon resonance technology to quantitate low-affinity binding (5a, 30).

As κ-light chains are 100-fold more abundant than λ-light chains in healthy animals and humans (21, 29), it is not surprising that we observed κ-light chains eluting from the cubilin affinity column but not λ-light chains. The current surface plasmon resonance data provides direct evidence confirming and extending our observation made by membrane binding of light chains: both κ- and λ-light chains are ligands for cubilin.

Studies of classic binding kinetics utilizing Scatchard analysis demonstrated several ligands competing with light chain for brush-border membrane binding. These ligands include lysozyme, insulin, cytochrome c, myoglo-
bin, and β₂-microglobulin (2, 3, 5). Competition by low-molecular-weight proteins raises the probability that cubilin is a multiligand receptor responsible for the endocytosis and cellular trafficking of a number of proteins normally filtered in the glomerulus and catabolized in the kidney, extending the role of this scavenger pathway receptor to such diverse phenomena as rhabdomyolysis and insulin metabolism. The multiple putative ligands for cubilin reflect the precedent set by other giant glycoprotein receptors such as the low-density lipoprotein (LDL) receptor, megalin, and the α₂-macroglobulin receptor, which bind many ligands with a spectrum of affinities at multiple binding sites (17, 18, 26). The recent cloning data that reveal multiple EGF repeats and CUB domains further strengthens this expectation.

Receptor kinetic studies have demonstrated that light chain binding to receptors in cultured proximal tubule cells is followed by endocytosis and ultimately by lysosomal degradation (3). The present observations suggest that cubilin is a receptor that can mediate endocytosis of light chains in renal proximal tubular cells. Nearly 90% of the λ-light chain binding was displaced by anti-cubilin antibody. In contrast, antimegalin antibody did not compete with the brush border binding of this light chain at all. This suggests that cubilin is the quantitatively major receptor for this λ-light chain. However, at maximal inhibitory concentration of the anti-cubilin antibody, ~10% of light chain remained bound to brush-border membranes, suggesting presence of additional binding site(s) for this light chain.

Anti-cubilin antiserum also inhibited endocytosis of light chains significantly. This further confirms that cubilin binding is followed by endocytosis of light chain. However, less than total inhibition of light chain endocytosis by anti-cubilin antibody indicates that this pathway may not be the exclusive endocytic pathway for light chains and that there may be alternate pathway(s) which can compensate partially when the cubilin-mediated pathway is blocked. It is also possible that our antibodies may be less than blocking functionally, and incomplete inhibition of endocytosis may be on this basis.

Importantly, binding of light chains to scavenger pathway receptors is not just a structural observation, as light chains had potent direct effects on endosomal fusion reconstituted in vitro. This provides further evidence for the hypothesis that receptors can change the fusion properties of membranes in which they reside (8). The current observations extend earlier findings, as in this instance, receptor-ligand interaction in vitro modulates the fusion cascade. Some ligands, such as, LDL are known to induce endocytosis of the ligand-receptor complex after binding. Endocytosis is thought to be dependent on the protein components of the final common pathway of fusion, which have largely been identified and cloned (4, 22, 23). This hypothesis linking disruptions in cell trafficking to cytotoxicity may provide new insights into nephrotoxicity of both myeloma light chains as well as other low-molecular-weight proteins.

Although the molecular structure of light chains is well known (16), receptors that mediate their endocytosis in the proximal tubule cells are not fully identified (2, 3). Our studies suggest an important role for cubilin in the renal handling of light chains. Cubilin has long been suggested to be in the same family of receptors as megalin, a giant receptor that belongs to a class of single transmembrane domain receptors, homologous to the LDL receptor, including multiple binding domains, some of which are EGF repeats (18, 19, 26). However, recent cloning data demonstrates that cubilin lacks a transmembrane domain, and therefore it is a peripheral membrane protein and not a transmembrane receptor like megalin (26). Thus it resembles other receptors that lack internalization signals which are cointernalized by means of other receptors, such as the glycosylphosphatidylinositol-anchored urokinase receptor, which is endocytosed by coupling of urokinase receptor-bound urokinase-inhibitor complex to LDL receptor-related protein (19). Direct binding data suggests that cubilin first binds to megalin on cell membranes, where cubilin-megalin complex is internalized simultaneously for endosomal trafficking (19). Recent cloning of cubilin demonstrated several structural features of the protein that clarify the observed binding interactions extensively. A group of developmental proteins, such as spermadhesin, talloid protein, and bone morphogenetic protein-1 are comprised of multiple EGF and CUB binding domains. Although sharing the same structural components, cubilin appears unique for at least three reasons. First, it differs structurally from the previously reported examples because of its size, the large number of CUB domains, and lack of proteinase module. Second, it is the only CUB domain protein described in mammals that is associated with cell differentiation and thus “indirectly” related to embryonic development. Third, cubilin is the first mammalian protein containing CUB domains that is essential for embryonic development (19). Cubilin has at least 35 binding domains, if one counts each of the EGF and CUB binding domains as a single site. Hence, the requirement for large excesses of ligand in competition experiments is not unexpected. The large number of CUB domains can explain how this glycoprotein can bind a large number of potential ligands with varied molecular size and structure including immunoglobulin light chains. Thus our studies not only identify light chains as ligands for this receptor but also raise the possibility that cubilin may have a role in the binding and endocytosis of other low-molecular-weight proteins that are normally filtered in the glomerulus and endocytosed in the proximal tubule cells of the kidney.

There are 13,500 new cases of myeloma annually in the United States, and 1–4 new cases per 100,000 of population worldwide (21, 29). Although the precipitation of light chains with Tamm-Horsfall protein to form casts in renal distal nephron segments has been defined down to specific peptide sequences (13), the molecular characteristics of receptors that mediate the
endocytosis of light chains in the proximal tubule have not been defined. Identification of the proximal tubular receptor for light chains extends and complements these observations. The proximal tubule determines the distal delivery of low-molecular-weight proteins by reabsorbing the bulk of filtered proteins including light chains. Many low-molecular-weight proteins induce injury to the proximal tubule, whereas others precipitate in the distal nephron. Both these mechanisms contribute to the pathogenesis of tubulointerstitial nephropathies associated with low-molecular-weight proteins, such as multiple myeloma (2, 3, 13, 27, 29). Proximal reabsorption of light chains is associated with Fanconi syndrome, necrosis, and tubular atrophy (2, 3, 29). Data presented here suggest that light chain cubilin interaction results in a potent inhibition of endosomal fusion in vitro, identifying a novel mechanism of proximal tubular cytotoxicity. Taken together with understanding of distant tubular cast formation, identification of major renal binding proteins for myeloma light chains in the proximal tubules will allow detailed characterization of the binding site between cubilin and light chains, as well as other nephrotoxic low-molecular-weight proteins. This adds to the necessary mechanistic data of all affected nephron sites for the rational design of agents to protect from nephrotoxicity caused by myeloma light chains (16) as well as other low-molecular-weight proteins.

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