Production of gastric intrinsic factor, transcobalamin, and haptocorrin in opossum kidney cells

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Brada, N., M. M. Gordon, J.-S. Shao, J. Wen, and D. H. Alpers. Production of gastric intrinsic factor, transcobalamin, and haptocorrin in opossum kidney cells. Am J Physiol Renal Physiol 279: F1006–F1013, 2000.—Opossum kidney epithelial cells were shown previously to synthesize and secrete two cobalamin (Cbl)-binding proteins, presumed to be haptocorrin (Hc) and transcobalamin II (TCII). The present study examines the hypothesis that renal tubular cells also produce intrinsic factor (IF), and this production provides an explanation for the presence of IF in urine. By using antisera raised against human IF and against TCII, the presence of TCII was confirmed, and that of IF discovered in the media of opossum kidney (OK) cells in culture. The apparent molecular weight of IF and TCII was 68 and 43 kDa, respectively. Immunoreactivity on Western blot of the putative IF protein was blocked by recombinant human IF. When proteins se-creted into the media were separated electrophoretically under non-denaturing conditions after binding with \(^{57}\text{Co})\text{Cbl}, a broad major band migrated at a relative front independently of recombinant IF or TCII, and probably represents Hc, as the Cbl binding is blocked by cobinamide. Small amounts of bound \(^{57}\text{Co})\text{Cbl} migrated in the position of both IF and TCII, when cobinamide was present. The presence of IF and TCII in OK cells was confirmed by immunohistochemistry. Specific reac-tivity for IF (blocked by recombinant IF) was found in proximal tubules of opossum kidney, but not in any other portions of the nephron, confirming the ability of anti-human IF antiserum to detect opossum IF. A 732-bp fragment of IF, nearly identical in sequence to rat IF, was isolated by RT-PCR from opossum kidney mRNA, and Western blot con-firmed the presence of IF protein. The presence of IF was also documented in rat kidney by isolation of an RT-PCR frag-ment, immunocytochemistry, and Western blot. IF should be added to the list of renal (proximal) tubular antigens that are shared by other epithelia.

Gastric intrinsic factor (IF) is a protein expressed in most adult mammals in gastric mucosa, but also in many other tissues (2). Within the stomach, more than one cell type may express IF, even in the same species (19, 38). In some species (e.g., dog) the pancreatic duct cells provide the major source of IF (3, 4, 23, 40). In these ectopic locations, IF secretion appears to respond to the regulatory signals appropriate for that organ, e.g., cholecystokinin for pancreatic duct stimulation (39). IF is expressed in some fetal tissues, e.g., amniotic fluid (42), although it is largely gone by parturition (12). The amniotic IF is fetal in origin (15), probably derives from fetal gastric mucosa (1, 30), and mediates cobalamin (Cbl) absorption by the fetal intestine (1). In addition, the anlage of the amniotic sac, the yolk sac, also produces cubilin, the IF-cobalamin (IF-Cbl) recep-tor (28, 33, 37), providing another means by which amniotic fluid Cbl could be absorbed.

The kidney is another tissue that expresses cubilin, and in which IF has been found bound to its brush border receptor (31). IF has been found in rat and human urine, but the source was not known, although it was assumed that it was filtered from the serum (31, 42). The role of cubilin in the renal brush border has been thought to include recovery of IF filtered into the urine. IF in the serum has been detected only in the rat, but the level is low (31). The epithelial cell of the proximal tubule of the kidney shares membrane antigens with many other tissues, including bile ducts, intestinal villi, epididymal tubules, allantochorionic cells, and exocrine cells of the pancreatic, salivary, and lacrimal glands (7, 27); however, gastric antigens have not been identified previously in the proximal tubule. The present study examines the hypothesis that urinary IF may derive from the proximal tubule, not from the serum. IF was identified in the media of opossum kidney (OK) cells, a cell line with characteristics of epithelium of the proximal tubule (21), and in native opossum and rat kidney. Previous identification without the use of immunologic identification suggested transcobalamin II (TCII) and haptocorrin (Hc) as the only two Cbl-binding proteins produced by OK cells (32). The present finding extends the range of tissue-sharing antigens with the proximal tubule epithelium, and could provide an explanation for some of the IF found in urine and renal brush border.

MATERIALS AND METHODS

Materials. OK cells were obtained from the American Type Culture Collection (ATCC; CRL 1840). Cells were grown on...
T-75 flasks or on polypolyethylene filters (Transswell, Corning Costar, Cambridge, MA) in Eagle’s modified minimal medium (Cellgro, Mediatech, Herndon, VA) with the addition of 10% FCS. Medium was changed every other day, and cells were grown 7 days postconfluence (~10 days after plating) for analysis of proteins in the medium. Fresh media without FCS were added 48 h before removal for analysis. Fresh opossum kidney, pancreas, and stomach was generously provided by Drs. Nathaniel Soper and Robert Underwood, Dept of Surgery, Washington University Medical School. Rat kidney was removed from Sprague-Dawley rats (Sasco, Omaha, NE) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta. [57Co]Cbl (300 nM) after anesthesia with methoxyflurane and saline perfusion via the thoracic aorta.

Sample preparation and Western blotting. Total RNA was prepared from frozen OK cell pellets, or from freshly isolated opossum kidney, pancreas, and stomach, or from rat kidney, by using an Rneasy Midi Kit (Qiagen, Valencia, CA). Media were removed, dialyzed, and either concentrated 40-fold by using Centriprep (Amicon, Beverly, MA), or concentrated 24-fold on Cbl-Sepharose columns (Sigma). The concentrated media (400 µl) were incubated with 100 µl of a 50% suspension of Cbl immobilized on 4% beaded agarose (Sigma) for 1 h at 4°C. The agarose was then washed 2× with 100 mM Tris·HCl, pH 7.5, with 2 mM sodium azide (13). The agarose pellet was then resuspended in 100 µl of disruption buffer (62.5 mM Tris·HCl, pH 6.8, 2% sodium dodecyl sulfate, 1.55 g/l of dithiothreitol, and 2.5% glycerol) and released by boiling for 5 min. Concentrated media samples were applied to 7.5% or 10% polyacrylamide gels (22), in either the presence or absence of the denaturing agent, SDS.

Opossum and rat kidney and opossum gastric mucosa (2.5g) were homogenized in 2.5 ml of PBS, pH 7.4, containing 1% N-P40, 12 mM sodium deoxycholate, and 0.1% sodium dodecyl sulfate, with 10% protease inhibitor cocktail for mammalian cell extracts (Sigma) added just before homogenization. After incubation on ice for 1 h, homogenates were spun in the microfuge at 4°C, and the supernatant was decanted and frozen until use. One milliliter of extract with ~60 mg of protein was incubated with 0.2 ml of washed Cbl-Sepharose beads (Sigma) for 1 h at 4°C, washed ×2 with Tris, pH 7.15, and resuspended in 2% SDS, 100 mM dithiothreitol (DTT), and 50 mM Tris, pH 6.8. After boiling and centrifugation, the agarose supernatant was concentrated to 60 µl, reboiled, and loaded on a 10% SDS gel.

Western blots were developed as previously described by using antiserum (1:3,000) raised against recombinant IF produced in the baculovirus system (13, 14). In some experiments the antiserum against IF (1:3,000 dilution) was incubated overnight at 4°C with 6.25 µg of recombinant IF. This mixture was added to an Immobilon-P membrane (Millipore, Bedford, MA), containing the separated and transferred proteins from the OK cell media. Recombinant IF and TCII produced in Pichia pastoris (43) were used as binding protein standards. Antiserum against human TCII used at 1:2,500 dilution was obtained from Robert Allen, (Univ. of Colorado, Denver, CO). Antiserum against the rat IF-Chl receptor and porcine Hc have been described previously (18, 36).

Nondenatured gel electrophoresis. OK cell media were grown until 7 days postconfluence. Fresh media without FCS were added 48 h before removal for analysis. Media was concentrated 40-fold as described above before analysis. Twenty nanograms of porcine Hc (Sigma), 4.6 ng of recombinant IF or TCII, or 14 µg of total protein in concentrated samples were incubated with labeled Cbl in the presence and absence of a 5,300-fold (Hc and IF), a 5,500-fold (TCII), and a 10,500-fold (media) molar excess of cobinamide to block Cbl binding to Hc (but not to IF or TCII). Protein-bound radioactivity was separated in a nondenaturing 7.5% acrylamide gel system identical to standard SDS-PAGE, except that no SDS was included in either the sample, stacking gel, or separating gel. After the dye reached the bottom of the gel, the unfixed gel was subjected to autoradiography.

Immunocytochemistry. This analysis was carried out by using the ABC biotinylation system (Vector Laboratories, Burlingame, CA) as described previously (38). Fixed tissues were embedded in paraffin and sectioned at 5-µm thickness. The slides were deparaffinized, rehydrated, and treated with 1% H2O2 in methanol. Slides were then blocked in PBS containing 5% BSA and 10% normal goat serum. Dilutions of the primary IF or TCII antiserum were used at 1:200 and 1:100, respectively, and the slides were incubated at 37°C for 60 min. The secondary antibody, goat anti-rabbit biotinylated IgG, was added at a dilution of 1:200 for 20 min at 37°C, after which the slides were washed in PBS and incubated with Vectastain ABC (reagents A and B at 20 µl/ml for 30 min at 37°C). 3,3′-Diaminobenzidine tetrahydrochloride [Sigma Fast DAB tablet (0.7 mg/ml) + H2O2 (20 mg/ml)] was added to the slide for 3–5 min or until color was evident. Normal rabbit serum as primary antiserum was used as a control, and showed no reaction. Recombinant IF for blocking reactivity was added at a concentration of 1–2 µg/ml.

RT-PCR. The forward primers used corresponded to bp 180–209 (CCC CGCGCATCCTGATTGCGCATGAA) and the reverse primer to bp 780–812 (CGGAATTTCCCT-GCTTAATCTCCTTGATATC) from rat IF (8), yielding an expected fragment of 732 bp. Reaction products were cloned into the vector pCR 2.1 TOPO (Invitrogen, Carlsbad, CA) and sequenced by using an Applied Biosystems Sequencer.

RESULTS

Identification of Cbl-binding proteins after SDS electrophoresis. OK cell media and homogenates were bound to Cbl-Sepharose, boiled to release the proteins, and electrophoresed on denaturing SDS gels. Figure 1 shows that cell lysates and media both contain a protein that reacts with IF antiserum. The secreted protein shows the expected size of ~68 kDa, whereas the intracellular protein ran with an apparent relative mass (Mr) of 55 kDa. The IF-Cbl receptor was detected only in the cell lysate (Fig. 1). TCII immunoreactivity in cell lysate and media corresponded with a protein of ~48 kDa, identical in mobility to recombinant human TCII (Fig. 2). No immunoreactive protein was found by using antiserum raised against porcine Hc. To confirm the presence of IF, media was concentrated without initial passage over a Cbl affinity column. Once again a protein of ~70 kDa was identified by Western blot (Fig. 3). When the blot was performed on conditioned media with antibody against IF and with purified recombinant human IF added, IF immunoreactivity was completely blocked (Fig. 3). Western blots on media not concentrated by affinity columns, and using antiserum raised against human TCII or against porcine Hc, revealed no immunoreactive bands (data not shown).

Identification of Cbl-binding proteins after nondenaturing electrophoresis. Concentrated recombinant and media proteins with bound [57Co]Cbl were separated on a gel with any SDS (Fig. 4). Porcine Hc migrated
as a broad band in the top half of the gel (lane 1), and the labeled Cbl was completely displaced by excess cobinamide (lane 2). Recombinant human IF showed a band that ran coincident with the Hc peak (lane 3), and Cbl binding to IF was not altered by excess cobinamide (lane 4). In the media one broad major band ran with a relative front (Rf) similar to porcine Hc and accounted for over 90% of the total binding activity in the media.

Fig. 1. Western blot of intrinsic factor (IF) and its receptor in media and lysates of opossum kidney (OK) cells. Media were recovered after 48-h incubation in the absence of FCS and concentrated 24-fold. Cells were scraped from a T-75 flask, and the pellet homogenized in 0.5 ml of radioimmunoprecipitation assay buffer. Ten percent SDS-PAGE gels were used without stacking gels (Sigma, St. Louis, MO). Ten microliters of the cell lysates and concentrated media were applied to the gels. Western blots were performed as described in MATERIALS AND METHODS. Left: rabbit antiserum against rat IF-cobalamin complex (Cbl) receptor (CR; 1:2,000). Right: rabbit antiserum against human IF (1:3,000).

Fig. 2. Western blot of transcobalamin (TC) II in OK cell media and lysate. Samples were obtained and analyzed as described in Fig. 1. Rabbit antiserum against human TCII was used (1:2,500). Human recombinant TCII (0.1 μg protein) was used as a standard.

Fig. 3. Western blot of IF in OK cell media. Media were recovered after 48-h incubation in the absence of FCS and were concentrated 40-fold. Left: two samples of OK cell media demonstrate 1 immunoreactive band identical with that of the major band of recombinant human IF (0.1 μg protein) added for comparison. The blot was incubated for 1 h at room temperature with antibody against IF (1:3,000 dilution). Right: the membrane was incubated with antiserum plus 6.25 μg of recombinant human IF (total vol 20 ml). No immunoreactive bands were detected. Antiserum against human recombinant IF was incubated overnight at 4°C with 6.25 μg of recombinant human IF to assess the absence of reactivity (final dilution 1:3,000).

Fig. 4. Identification of cobalamin binding proteins in OK cell media on nondenaturing polyacrylamide gel. Polyacrylamide electrophoresis in 7.5% gels was performed to separate Cbl-binding proteins in concentrated media from OK cells as described in MATERIALS AND METHODS. Purified recombinant (human IF, TCII) and native (porcine haptocorrin (Hc)) binding proteins and conditioned media from OK cells were mixed with labeled Cbl in the presence and absence of a molar excess of cobinamide (Cob; 5,300-fold for Hc and IF, 5,500-fold for TCII, and 10,500-fold for media). The affinity column-concentrated media (14 μg protein) and purified Cbl-binding proteins (4.6 μg protein) were separated on a nondenaturing acrylamide gel, as described in MATERIALS AND METHODS. The migration of porcine Hc alone and with added Cob is shown in lanes 1 and 2. Lanes 3–8 show binding of IF, OK cell media, and TCII without (lanes 3, 5, 7) and with (lanes 4, 6, 8) added Cob. Note that Cob completely blocks the binding of [57Co]Cbl (lane 2) but does not block binding to IF or TCII (lanes 4 and 8). Note the wide radioactive band in the media [relative front (Rf) 0.16–0.40], by using the fast-moving free Cbl band as the leading edge (lane 5) that is largely blocked by Cob (lane 6). However, a band of radioactivity persists at the Rf of IF (0.3). The minor faster moving band with Rf of 0.64 corresponds with TCII, and this shows up faintly in the media as well (lane 6). The leading band of radioactivity corresponds with free Cbl.
The diffuse nature of the band is consistent with behavior of Hc in other systems, due to its extensive glycosylation, and probably represents opossum Hc. When conditioned media was incubated with cobinamide, one band of radioactivity remained (lane 6), unlike the incubation with porcine Hc alone (lane 1). This band corresponded with the Rf of IF, and probably represents opossum IF secreted into the OK cell media. Recombinant TCII labeled with Cbl runs closer to the electrophoretic front (lane 7), and retains its label in the presence of cobinamide (lane 8). A labeled protein of similar Rf was seen in conditioned media with and without cobinamide (lanes 5 and 6) and accounts for only a small percent (6%) of Cbl binding activity in the media. The absence of SDS in the gel allows determination of Cbl binding, but did not provide sufficiently concentrated bands for detection of IF and TCII by Western blotting.

**Immunocytochemistry.** Figure 5 shows that OK cells stained positively for both IF (A) and TCII (B), whereas no staining was seen with normal rabbit serum (D). The immunoreactivity was weaker for IF than for TCII, consistent with the 2-fold greater Cbl binding of TCII vs. IF in OK cell media (Fig. 4). The stain was seen in the cytoplasm and appeared more concentrated near the apical surface of the cells. Recombinant IF was added to the media for 1 h at 37°C to produce a pattern that might be expected when proximal tubular cells absorb IF from the urinary lumen. In this instance (Fig. 5C) the stain was found much more on the apical surface of the cells, and the cytoplasm was more deeply stained than by endogenous IF alone (A).

Previous studies failed to detect immunoreactive IF in OK cells (32), and the reaction in the present study was also weak. Figure 6A demonstrated strongly positive IF staining in proximal collecting tubules (PCT) present in opossum kidney PCT. The distal tubules were not reactive (arrows). This staining was blocked completely by the addition of recombinant human IF (Fig. 6B) showing that opossum IF is readily detected by anti-human IF antibody. Thus the weak response in OK cells (Fig. 5) is probably related to limited production by those cells and not to decreased antibody reac-

![Fig. 5. Immunocytochemical identification of IF and TC in OK cells. The cells were grown on Transwell filters until 7 days postconfluence. A: antiserum against human IF (1:200). B: antiserum against human TC (1:200). C: antiserum against human IF (1:200) after incubation of the cells with recombinant human IF (17 μg/ml). D: normal rabbit serum (1:200).](http://ajprenal.physiology.org/)

![Fig. 6. Immunocytochemical identification of IF in normal opossum kidney. A: antiserum against human IF was applied at 1:200 dilution. Note the restriction of IF reactivity to the proximal collecting tubule. Rat kidney, but not opossum kidney, shows decreased IF reactivity in the distal tubule. B: recombinant human IF at 1.7 μg/ml was added to the antiserum. Note the absence of immunoreactivity. PCT, proximal collecting tubules; DCT, distal tubule. Magnification ×125.](http://ajprenal.physiology.org/)
tivity. Addition of recombinant human IF blocked reactivity in rat kidney as well (compare B and D with A and C, Fig. 7). However, in the rat kidney the distal tubules (arrows) also contained IF, although much less than the proximal tubules (arrowheads). In the intact kidneys of both species, the immunoreactive precipitate could identify IF either endogenously produced or IF endocytosed from the lumen, or both. The absence of immunostaining in the apical membranes favors endogenous IF as the source of positive staining.

Identification of endogenous renal IF. RT-PCR was performed to demonstrate that IF was indeed produced endogenously. RT-PCR reactions produced a product of the appropriate size (732 bp) by using mRNA from opossum kidney and pancreas, but not from stomach, under the conditions used (Fig. 8A). The sequence of 190 internal amino acids obtained showed 99% identity (188/190) with that of rat IF, with the exception of C527T, resulting in amino acid change F164S, and G688A, corresponding to D218N, according to the numbering assignments of the rat-cDNA clone (8). RT-PCR reactions with the same primers produced a similar-sized fragment by using mRNA from OK cells (data not shown) and from rat kidney (Fig. 8B). In the rat, the stomach contains the largest amount of IF mRNA (Fig. 8B); however, IF mRNA was clearly seen in rat kidney.

Western blots were performed on affinity column-concentrated extracts of opossum stomach and kidney and rat kidney (Fig. 9). In the opossum stomach, faintly positive bands were seen, corresponding to apparent \( M_r \) of 62, 70, and 95 kDa (Fig. 9A). Three bands were also seen in opossum and rat kidney extracts (Fig. 9B), consistent with a similar pattern seen previously in rat stomach (23). The two upper bands in opossum and rat kidney were identical in size with those in opossum stomach, but the smallest band was somewhat smaller, and a doublet was found in the opossum kidney.

DISCUSSION

The production of IF by (proximal) renal tubular cells provides an explanation for the presence of some IF in the urine. In fact, all three Cbl-binding proteins (IF, TCII, and Hc) appear to be produced by these cells, and all have been found in human urine (42). Consis-
tent with this finding, the Cbl-binding proteins are preferentially secreted into the apical media by OK cells; however, they also appear in the basolateral media (32). About one-half of the Cbl binding activity in human urine was blocked by cobinamide and might represent Hc (42). Although the contribution of IF to the total Cbl binding in OK cell media was low, the data on Cbl binding in human urine suggest a larger contribution from IF.

It has been suggested that some of the IF in urine might derive from filtration of serum IF. Two IF reactive polypeptides (62 and 50 kDa) were found in rat serum, but intravenously administered [125I]IF was taken up rapidly by an uncharacterized receptor in the liver, and was not reported to appear in urine to account for the 80–170 pg of IF secreted daily in urine (31). Although IF was detected by immunoblot in the rat renal cytosol and Golgi, it was suggested that serum was the origin of the urinary IF (31). Serum IF might result from absorbed IF that escapes lysosomal hydrolysis after endocytosis in the ileum. However, intact IF does not appear to survive transcytosis across

Fig. 8. RT-PCR-derived fragments from opossum and rat tissue mRNA. A: the opossum kidney and pancreas contained the predicted 732-bp fragment, but opossum stomach did not, under the conditions used. Sequencing 1 fragment from each tissue revealed near identity with rat IF sequence (8). B: the rat kidney fragment was identical in size to that from the more abundant gastric one. Sequence from both fragments revealed rat IF.

Fig. 9. Western blots of extracts from opossum and rat tissues. A: opossum stomach; B: opossum and rat kidney. Tissue extracts were obtained and concentrated by using Chl-Sepharose as described in MATERIALS AND METHODS. Antibody against human recombinant IF was used at 1:3,000 dilution.
intestinal cells in culture (14, 32). Alternatively, IF might reach the blood by direct secretion from gastric mucosal cells (19) or from the proximal and distal tubule of the kidney. The present data showing IF mRNA, cytosolic staining in rat kidney, and Western blot identification in kidney extracts are consistent with the cytosolic and Golgi distribution of IF reported previously (31).

Several explanations have been offered to account for the variation between ileal and urinary IF-Cbl receptor activity in patients with Grasbeck-Imerslund disease, a syndrome characterized by Cbl deficiency, the cause of which is decreased Cbl absorption across the intestinal mucosa, although IF production is normal (17). Urine has been used as a source of receptor fragments for a radioisotope binding assay to detect the IF-Cbl receptor (8, 18). The values for IF-Cbl binding to a receptor fragment in the urine have been low in cases of Grasbeck-Imerslund disease, a syndrome in which Cbl absorption from the intestine is decreased. Yet, two recent case reports show an elevated ileal level of the IF-Cbl receptor (11). In addition, increased IF has been reported in the urine of some Finnish patients with Grasbeck-Imerslund syndrome (10). This result was interpreted as confirmation of the low IF-Cbl receptor activity in those patients. The present data are consistent with the interpretation that varying levels of IF in the urine, originating from the kidney itself, might combine with Cbl and inhibit binding of labeled IF-Cbl to urinary protein, thus producing unexpected values for the IF-Cbl binding assay.

Both IF and TCII sequences are well preserved between rodents and humans (2, 24). Amplification of IF gene expression occurs after weaning in the rat and is not observed in the distal tubule (41). Opossums hold a position on the phylogenetic tree midway between rodents and chickens (5, 26). Analysis of cloned sequences of many proteins reveal identity of 65–91% with rat and mouse sequences, and 54–81% with human ones (6, 24, 29, 35). One would expect that some cross-reaction with antiserum raised against human ones (6, 24, 29, 35). One would expect that some cross-reaction with antiserum raised against human IF and TCII receptors. The differences from other species for opossum TCII might be greater than for IF, as conservation of TCII primary structure may not be so great. For example, not all species have a structure for TCII that allows binding to Quso under the same conditions as for human TC (20).

The present data show, surprisingly, that OK cells expressed IF as well as TCII, in addition to Hc. The antiserum against porcine Hc did not identify an opossum counterpart. The same negative result has been found by using antiserum against human Hc (32). However, the majority of Cbl binding capacity secreted from OK cells would appear to represent Hc, as it migrates on native gels with a broad Rf that does not correspond to the Rf of TCII, and overlaps the Rf of IF. The presence of the IF-Cbl receptor suggests that OK cells were sufficiently differentiated to express the expected membrane antigens shared with other tissues. The Mₚ of the receptor was only about one-half (110 kDa) that of the receptor isolated from opossum kidney (34). Proteolytic degradation might have occurred during the processing of cells to demonstrate the OK cell receptor. IF was found at the expected Mₚ by Western blotting in denaturing gels. In nondenaturing gels Cbl binding activity was found in OK cell conditioned media at the Rf of recombinant IF, when Hc binding of Cbl was blocked by cobinamide. PCR from RNA isolated from opossum kidney, pancreas, and OK cells detected an internal sequence nearly identical to rat IF. In addition, IF mRNA was found by PCR in rat kidney, and Western blots on opossum stomach and kidney and on rat kidney extracts identified the pattern of three IF-reactive proteins reported previously in rat gastric extracts (23). Therefore, IF should be added to the list of antigens expressed by the proximal tubule, but which are not kidney specific (7, 27).

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REFERENCES


