FcRn-mediated transcytosis of immunoglobulin G in human renal proximal tubular epithelial cells

Noriyoshi Kobayashi\textsuperscript{1,2}, Yusuke Suzuki\textsuperscript{1,2}, Toshinao Tsuge\textsuperscript{1,2}, Ko Okumura\textsuperscript{2}, Chisei Ra\textsuperscript{2}, Yasuhiko Tomino\textsuperscript{1}

\textsuperscript{1}Division of Nephrology, Department of Internal Medicine,
\textsuperscript{2}Atopy (Allergy) Research Center,
Juntendo University School of Medicine, Tokyo 113-8421, Japan

Correspondence should be addressed to Dr Yasuhiko Tomino,
Division of Nephrology, Department of Internal Medicine,
Juntendo University School of Medicine,
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
Tel: +81-3-5802-1065, Fax: +81-3-5802-1065
E-mail: yasu@med.juntendo.ac.jp

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Running title: FcRn-mediated IgG transcytosis in RPTECs
Abstract

In the kidney, proteins filtered through glomeruli are reabsorbed by endocytosis along the proximal tubules to avoid renal loss of large amounts of proteins. Indeed, various proteins such as albumin, β2- microglobulin and several hormones are reabsorbed via receptor-mediated endocytosis in the proximal tubules. Recently, neonatal Fc receptor (FcRn) that is involved in the transport of IgG across several epithelial and endothelial cells was reported to be expressed in renal proximal tubular epithelial cells. It was suggested that FcRn may play an important role in the reabsorption of IgG from the tubular fluid. However, to date there has been no direct evidence for receptor-mediated endocytosis of IgG in the renal proximal tubular epithelial cells in humans. To explore physiological roles of FcRn in the proximal tubules, we examined IgG transport using the human renal proximal tubular epithelial cells (RPTECs). FcRn was expressed in RPTECs and physically associated with β2- microglobulin, preserving the capacity of specific pH-dependent IgG binding. Both cell surface and intracellular constitutive expression of FcRn was detected with a specific antibody to FcRn by immunofluorescence. Human IgG was bound to the cell surface of RPTECs in a pH-dependent manner. The human IgG transport assay revealed that receptor-mediated transepithelial transport of intact IgG in RPTECs is bidirectional and that it requires the formation of acidified intracellular compartments. Using double immunofluorescence, the internalized human IgG was marked in cytoplasm of RPTECs and co-localized with FcRn. These data define the mechanisms of FcRn-associated IgG transport in the RPTEC monolayers. It was suggested that the intact pathway for human IgG transepithelial transport may avoid lysosomal degradation of IgG.
Key Words: neonatal Fc receptor, IgG transport, proximal tubule, IgG homeostasis, mucosal immunity
Introduction

After blood filtration by glomeruli, filtrated plasma proteins are reabsorbed via the endocytic pathway by renal proximal tubules. Albumin, β₂-microglobulin which are known as low molecular weight plasma proteins, and several hormones are reabsorbed via the receptor-mediated endocytic pathway by the proximal tubules (1). Reabsorption of these proteins via receptor-mediated endocytosis and their consecutive delivery to lysosomes for degradation in the proximal tubules may play important roles in protein and hormonal homeostasis (1). Under normal conditions, urinary protein includes approximately 40% albumin, 5 to 10% IgG, 5% immunoglobulin light chains, 3% IgA, and other proteins (1, 2). In glomerular diseases, a large amount of filtered plasma protein is followed by increased reabsorption in the proximal tubules and causes progression of chronic renal diseases (3, 4). Several studies suggest that reabsorption of albumin in the proximal tubular cells may be mediated by specific receptor-mediated endocytosis, and excessive reabsorption of albumin may induce expression of numerous pro-inflammatory genes (5, 6). Immunoglobulins are also presented in urine under intact or impaired renal conditions. Some studies reported that immunoglobulin might induce tubular damage similar to other proteins (4, 7), but details of this mechanism are not yet clear.

In the kidneys, receptor-mediated transport of immunoglobulins has been studied in the polymeric immunoglobulin receptor, which transports polymeric IgA and IgM from the basolateral to the apical surface (8, 9). This receptor-mediated transport of polymeric IgA plays an important role in mucosal immunity of the urinary tract (10, 11). Other studies reported receptor-mediated endocytosis of the immunoglobulin light chain (12, 13), but the precise steps involved in IgG endocytosis and catabolism by the
proximal tubular cells are still unknown.

On the other hand, receptor-mediated endocytosis of IgG has been extensively studied in passive immunity from mothers to their young. This receptor is known as the neonatal Fc receptor (FcRn) and was initially identified in rodents as the receptor which mediates the transport of maternal immunoglobulins to the young via the neonatal intestine (14, 15). FcRn is associated with β2-microglobulin and is structurally homologous with the α-chain of the MHC class I molecule (16). One of the specific characteristics of FcRn is pH-dependent IgG binding, that is high-affinity binding at acidic pH and weak or no binding at neutral pH. IgG is transported to the fetus or neonate across the intestinal epithelium or yolk sac in rodents (14-16), and across the placenta in humans (17-20). It has been suggested that FcRn may play a critical role in the passive immunity (14-20). More recently, several studies indicated that this receptor is not only implicated in the transport of maternal IgG to the young for passive immunity, but also in maintenance of IgG homeostasis by recycling internalized IgG beyond the neonatal period (21, 22). Since internalized IgG that binds to FcRn is prevented from degradation in lysosomes, intact IgG may cross the epithelia.

Recently, Haymann et al. reported that FcRn was expressed in the human renal glomerular epithelial cells and brush borders of the proximal tubular cells (23). They suggested that FcRn in proximal tubular cells may mediate endocytosis of IgG and play a role in the reabsorption of IgG from the tubular fluid, but the physiological function of FcRn has not been clarified. In the present study, the functional expression of FcRn and the endocytic pathway of IgG were examined in human RPTECs.
Materials and Methods

Cells and cell culture conditions

Human RPTECs were purchased from Clonetics, Inc. (San Diego, CA). Identity and purity of RPTECs were examined by staining of γ-GTP, and phase contrast microscopy. RPTECs were cultured on collagen coated plastic dishes and studied at the third to the fifth passage in Renal Epithelial Cell Growth Medium (Iwaki Glass, Tokyo, Japan) containing 50 μg/ml gentamicin, 50 ng/ml amphotericin B, 10 μg/ml transferrin, 5 μg/ml insulin, 10 ng/ml human recombinant epidermal growth factor, 500 ng/ml epinephrine, and 0.5% fetal bovine serum. For transport studies, approximately $3 \times 10^4$ cells/cm$^2$ were seeded on the collagen coated Transwell-Clear polyester membrane inserts with a diameter of 6.5 mm and pore size of 0.4 μm (Corning, Tokyo, Japan) to obtain polarized cell monolayers as previously described in a rat kidney cell line (24). The presence of a continuous monolayer was routinely monitored by microscopic evaluation of all filters. The transepithelial electrical resistance (TEER) measured by Millicell-ERS (Millipore Corp., Bedford, MA) attained stable levels after 7 days (data not shown).

Human intestinal epithelial cell line T84 cells were purchased from American Type Culture Collection (Manassas, VA) at passage 52 and maintained in a 1:1 mixture of F12K and DMEM containing 5% FCS. Since U937 cells express a low amount of FcRn, we transfected a full-length cDNA encoding hFcRn into U937 cells and used them as positive controls. U937 cells transfected with or without the full-length cDNA encoding hFcRn were maintained in RPMI 1640 medium containing 10% FCS. Jurkat cells were also maintained in RPMI 1640 medium containing 10% FCS and used as negative controls.
RNA preparation and reverse transcription-PCR (RT-PCR)

Total RNA was isolated from RPTECs, T84, PBMC and U937 cells using Trizol (Life Technologies, Rockville, MD). Total RNA (2 µg) was converted to cDNA using oligo(dT) primers (Life Technologies, Rockville, MD) and reverse transcriptase (Superscript Ⅱ, Life Technologies, Rockville, MD). The single-strand cDNA product was denatured and amplified in a GeneAmp PCR System 9600 (PerkinElmer, Norwalk, CT) with each set of primers chosen on the basis of the human FcRn (hFcRn), FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) sequences (18, 25-27). The regions amplified by each set of primers were as follows: FcRn nucleotides 255 to 807, 5’-ACT CCT GCC TTC TGG GTG TC-3’, 5’-GGT AGA AGG AGA AGG CGC TG-3’; CD16 nucleotides 282 to 393, 5’-CAG TGG AGA GTA CAG GTG CC-3’, 5’-CTC CTT GAA CAC CCA CCG AG-3’; CD32 nucleotides 121 to 545, 5’-CCC AAA GGC TGT GCT GAA AC-3’, 5’-GTG GTT TGC TTG TGG GAT GG-3’. Two primers specific for CD64 cDNA were identical to those described previously (25). The PCR products were separated by electrophoresis on 2.0% agarose gels and visualized by ethidium bromide staining. The fidelity of the PCR products was also confirmed by nucleotide sequencing.

Affinity-purified rabbit anti-hFcRn antibody

Rabbit polyclonal anti-hFcRn antibody against the peptide was raised in New Zealand White rabbits. The peptide consisted of amino acids 112-125 of the α2 domain of hFcRn, plus an N-terminal Cys for conjugation (Sawady Technology Co. LTD., Tokyo, Japan) (17). Gene bank searches indicated that the sequence of the peptide was unique to hFcRn and showed no similarities to other molecules. Specific rabbit anti-hFcRn antibodies were isolated from immune serum by affinity purification on the peptide
immobilized to a Sulfolink matrix (PIERCE, Rockford, IL) according to the manufacturer’s instructions. The antibodies worked well in Western blot analyses, immunoprecipitation and immunostaining.

**Immunoblot analysis**

RPTECs, Jurkat cells and U937 cells transfected with a full-length cDNA encoding hFcRn (U937-hFcRn) were extracted in 1% Nonidet P-40 in PBS. Protein concentrations in the extracts were determined by the bicinchoninic acid method (BCA, PIERCE, Rockford, IL) with BSA standards. The extracts were resolved on 12.5% SDS-PAGE, transferred onto the PVDF membrane (Japan Millipore, Yonezawa, Japan) and probed with a rabbit anti-hFcRn antibody (1 µg/ml) or pre-immune serum. After incubation with a second HRP-conjugated goat anti-rabbit IgG antibody (Cappel, ICN Pharmaceuticals, Inc., Aurora, OH), the signal was detected by an enhanced chemiluminescence system (ECL-plus, Amersham Pharmacia Biotech, Buckinghamshire, UK) and visualized by FUJIFILM luminescent image analyzer LAS-1000 plus.

**Immunoprecipitation**

RPTECs, Jurkat cells and U937-hFcRn (5 × 10⁶) were lysed in 5 mg/ml CHAPS in 50 mM phosphate buffer containing protease inhibitors (Complete™, Boehringer Mannheim GmbH, Mannheim, Germany). After centrifugation, the supernatant was collected and pre-cleared with 30 µl of protein G-Sepharose 4FF beads (Amersham Pharmacia Biotech, Buckinghamshire, UK). The lysates were then subjected to immunoprecipitation with a rabbit anti-hFcRn antibody or pre-immune serum. The immunoprecipitates were collected on protein G-Sepharose 4B beads, washed with the lysis buffer, and extracted from the beads by boiling in 60 µl sample buffer containing
2-mercaptoethanol (2-ME). Then 20 µl of each sample was analyzed by SDS-PAGE (5-20% gradient gel; BC BIO CRAFT, Tokyo, Japan), transferred onto the PVDF membrane and probed with a goat anti-human β₂-microglobulin antibody (Nippon Bio-Test Laboratories Inc., Tokyo, Japan).

**pH-dependent binding of cell lysates to human IgG-agarose**

Affinity binding of hFcRn to human IgG-agarose was carried out as previously described with some modifications (19, 20, 28). Briefly, 6 × 10⁶ RPTECs were lysed with 5 mg/ml CHAPS containing protease inhibitors in 50 mM phosphate buffer adjusted either to pH 6.0 or pH 8.0. The solubilized total proteins were incubated with 60 µl of human IgG-agarose corresponding to 300 µg of IgG at 4°C for 12h. The IgG agarose beads were collected by centrifugation and washed with lysis buffer (pH 6.0 or pH 8.0). Bound proteins were eluted by boiling in sample buffer and analyzed by 12.5% SDS-PAGE under reducing conditions. For Western blot analysis, proteins were transferred onto the PVDF membrane and probed with a rabbit anti-hFcRn antibody and then re-probed with a goat anti-human β₂-microglobulin antibody.

**Human IgG binding assay**

Biotinylated human IgG (Vector Laboratories, Inc., Burlingame, CA) binding assay was carried out as previously described (23) with some modifications. Briefly, RPTECs grown on 100 mm diameter type I-collagen coated culture dishes (Iwaki Glass, Tokyo, Japan) were detached with 5mM EDTA and resuspended in binding buffer (Hanks’ balanced salt solution, with 10 mM Hepes, pH 6.0 or 8.0, containing 0.1% BSA). The cells were pelleted, washed, and then resuspended in the binding buffer at 1 × 10⁶ cells/ml. The cell suspension was mixed with biotinylated human IgG (10 µg/ml), with or without 1mg/ml unlabeled human IgG. After incubation at 4°C for 4h on a rotating
mixer, the suspensions were spun down and unbound ligands were removed by washing with the binding buffer of pH 6.0 or pH 8.0. The cells were then spun down and dissolved in 1% Nonidet P-40 in PBS. The same quantity of proteins was applied to 12.5% polyacrylamide denaturing gels, transferred onto the PVDF membrane and probed with a HRP-conjugated avidin. The signal intensity of the bands was analysed by FUJIFILM luminescent image analyzer LAS-1000 plus.

**Human IgG transport assay**

Human IgG transport assay was performed as previously described (29) with some modifications (20, 24, 28). Briefly, RPTEC monolayers exhibiting stable electrical resistance were washed and equilibrated in Hanks’ balanced salt solution with 10 mM Hepes, pH 7.4, containing 0.025% BSA. Biotinylated human IgG or IgY (Sigma Chemical Co., Saint Louis, MO) was added at 100 µg/ml to the apical or basolateral chamber. Unlabeled IgG or IgY (10 mg/ml) was used as a competitive inhibitor. To evaluate the effects of pharmacological agents that interfere with cell trafficking, RPTEC monolayers were pre-treated with 0.1 µM bafilomycin A1 (Sigma Chemical Co., Saint Louis, MO) which alkalinizes endocytic vesicles by specifically inhibiting the vacuolar proton pump (29, 30). RPTECs were incubated with ligands at either 37°C or 4°C, and contralateral chamber medium was collected at various times. Transported proteins were concentrated with Centricon YM-100 (Millipore Corp., Bedford, MA) and analyzed by SDS-PAGE and ligand blot after reduction with 2-ME. The signal intensity of the bands was compared using FUJIFILM luminescent image analyzer LAS-1000 plus and measured against control biotinylated human IgG (12.5 ng biotinylated human IgG standard).
**Immunofluorescence**

RPTECs grown on glass coverslips were incubated for 1h at 4°C in binding buffer (0.05% BSA, Hanks’ balanced salt solution, with 10 mM Hepes, pH 6.0 or 8.0), containing 1 mg/ml human IgG. The cells were then washed with PBS at pH 6.0 or pH 8.0 to remove non-bound human IgG. Thereafter, the cells were incubated at 37°C for another 30 minutes to allow internalization of the bound human IgG in binding buffer (pH 6.0 or pH 8.0). Following internalization, the cells were washed, fixed with 2% formaldehyde and 4% sucrose in PBS, and then permeabilized with 0.3% Triton X-100 in PBS. After blocking with 2% BSA, 2% FCS and 0.2% fish gelatin in PBS for 30 min, FITC-conjugated goat anti-human IgG antibody (1: 100)(Jackson ImmunoResearch, West Grove, PA) was applied and incubated at room temperature for 1h. Co-localization of the internalized human IgG with hFcRn was detected using rabbit anti-hFcRn antibody (1: 50) and rhodamine-conjugated goat anti-rabbit IgG antibody (1: 100) (Cappel, ICN Pharmaceuticals, Inc., Aurora, OH). To detect the cell surface expression and steady state distribution of hFcRn, RPTECs grown on glass coverslips were fixed with or without permeabilization using 0.3% Triton X-100 in PBS. Staining procedures to detect hFcRn were the same as described above. The fixed cells were mounted in IMMUNON (SHANDON, Pittsburgh, PA) and then viewed using a Bio-Rad MRC-1024 confocal microscope.

These experiments were reproduced at least three times by independent studies.
Results

Expression and physical association of hFcRn and \( \beta_2 \)-microglobulin in RPTECs

Specific mRNA for the FcRn was expressed in RPTECs by RT-PCR as shown in Fig. 1A. PCR products of the expected size (553bp) were obtained from RPTECs, and T84 cells, a human intestinal epithelial cell line expressing functional hFcRn (positive control). To further confirm whether the detected hFcRn mRNA was translated to the detectable protein, Western blotting using RPTECs, Jurkat and U937-hFcRn cell extracts was performed. As shown in Fig. 1B, a single specific band of approximately 45 kDa was detected in RPTECs (lane 1), the same as in U937-hFcRn (lane 3) but not in Jurkat cells (lane 2), using rabbit anti-hFcRn antibody. These bands were not detected with the pre-immune serum (lanes 4, 5 and 6). Since hFcRn associates with \( \beta_2 \)-microglobulin, immunoprecipitation of the cell lysates using the same antibody was performed as Western blotting analysis (Fig. 1C). Approximately 12 kDa bands were detected in RPTECs (lane 3), U937-hFcRn (lane 1) and purified human \( \beta_2 \)-microglobulin (lanes 4 and 8) but not in Jurkat cells (lane 2). These bands were not detected when immunoprecipitated with the pre-immune serum (lanes 5, 6 and 7). To determine whether FcRn expressed in RPTECs preserves specific pH-dependent binding capacity for IgG, the cell lysates incubated with human IgG-agarose in pH 6.0 or pH 8.0 were analyzed by Western blotting. As shown in Fig. 1D, hFcRn preferentially bound to human IgG-agarose at pH 6.0 (lane 1) and coprecipitated with \( \beta_2 \)-microglobulin (lane 3), but the binding was significantly reduced at pH 8.0 (lane 2). It was assumed that two other bands of lanes 3 and 4 were nonspecific reactions with heavy and light chains of human IgG. hFcRn protein was expressed in RPTECs and physically associated with \( \beta_2 \)-microglobulin, and it preserved specific pH-dependent
IgG binding.

**Cell surface and intracellular expression of hFcRn in RPTECs**

As shown in Figs. 2A and 2B, both cell surface and intracellular expression of hFcRn was detected with the specific antibody. No staining was observed when RPTECs were incubated with pre-immune serum and second antibodies (Figs. 2C and 2D). hFcRn was constitutively present on the plasma membrane and in the cytoplasm of RPTECs.

**No detection of FcγRI, FcγRII and FcγRIII transcripts in RPTECs**

As shown in Fig. 3, PCR products of the expected size were detected as follows; 112bp fragment specific for CD 16 in the PBMC (lane 1), 425bp fragment specific for CD 32 (lane 6) and 600, 880bp fragment specific for CD64 (lane 6) in U937 cells but not in RPTECs (lanes 3 and 8). RT-PCR amplification of human GAPDH provides an internal control for each reaction (lanes 1, 3, 6 and 8). There was no genomic DNA contamination because RT-PCR performed on each RNA without reverse transcriptase yielded negative results (lanes 2, 4, 7 and 9).

**Specific pH-dependent human IgG binding to the cell surface of RPTECs**

As shown in Fig. 4, biotinylated human IgG could bind to RPTECs in a specific manner at pH 6.0, and binding of biotinylated human IgG was significantly reduced in the presence of excess amounts of unlabeled IgG at pH 6.0. In contrast, binding levels of biotinylated human IgG were much lower at pH 8.0. The specific pH-dependent IgG binding proteins were expressed on the plasma membrane of RPTECs.

**Receptor-mediated transcytosis of human IgG in RPTECs**

FcRn-dependent IgG transport in RPTEC monolayers by transepithelial flux of biotinylated human IgG using transwell inserts was investigated. Biotinylated human IgG was added to the apical or basolateral chamber of the cell culture inserts at 4°C or
37°C. As shown in Figs. 5A and 5B, intact human IgG was transported in both directions. Transport of human IgG was detected in monolayers incubated at 37°C but not in those incubated at 4°C (data not shown). Chicken IgY was not transported at detectable levels in either direction (data not shown). To further confirm whether this receptor-mediated transcytosis was specific for IgG, we analyzed biotinylated human IgG transport with excess unlabeled human IgG or chicken IgY as competitive inhibitors. Bidirectional transcytosis of biotinylated human IgG was significantly reduced in the presence of excess unlabeled IgG (Figs. 5A and 5B). In contrast, excess chicken IgY did not compete with biotinylated human IgG (Figs. 5A and 5B). The receptor-mediated transport was specific for IgG. To evaluate the effects of pharmacological agents that interfere with acidification of the endosomes, RPTEC monolayers were pretreated with bafilomycin A1, specific inhibitor of H⁺-ATPase. Bidirectional transcytosis of biotinylated human IgG was significantly reduced by pretreatment with bafilomycin A1 (Figs. 5A and 5B), suggesting that the receptor-mediated transepithelial transport of IgG in RPTECs requires acidified intracellular compartments.

**Co-localization of the hFcRn and internalized human IgG in RPTECs**

To identify the pH-dependent IgG binding protein as hFcRn, we visualized the localization of hFcRn and internalization of human IgG in RPTECs. As shown in Fig. 6A, human IgG bound to the RPTEC cell surface at pH 6.0 was internalized after incubation at 37°C. hFcRn was also detected in cytoplasm with a rabbit anti-hFcRn antibody (Fig. 6B). Merging of the two panels showed extensive co-localization of hFcRn and human IgG (Fig. 6C). In contrast, internalized IgG was not detected in RPTECs when pre-incubated with human IgG at pH 8.0 (Fig. 6D) in spite of hFcRn
expression (Fig. 6E). hFcRn mediated transcytosis of human IgG in RPTECs.
Discussion

The endocytic function of renal proximal tubular epithelial cells has been studied with various proteins which are present in the urine (1, 31). Albumin, the most prominent protein in glomerular filtrate, is reabsorbed by proximal tubules via specific receptor-mediated endocytosis (5, 32). Although the reabsorption of albumin prevents the loss of large amounts of this major plasma protein via the urine, excess reabsorption of albumin may be a factor for the development and progression of chronic renal diseases (5, 6). Thus, the kidney appears to have a limit on the amount of protein which can be reabsorbed and catabolized at the greatly increased filtered-loads found in renal diseases.

On the other hand, immunoglobulins such as IgG, IgA, and their fragments including free light chains and Fc portions of IgG heavy chains have also been found in the urine (1). Similar to other proteins, absorption of these molecules seems to be regulated by the endocytosis pathway in the proximal tubules, but the mechanisms are not fully understood (33, 34). Therefore, the clarification of the endocytosis pathway of these proteins in the proximal tubular epithelial cells is important physiologically and pathophysiologically.

In the present study, we examined whether FcRn, which is substantially involved in IgG transport in other tissues (21), is functionally expressed in human renal proximal tubular epithelial cells and mediates endocytosis of IgG. FcRn was actually expressed in RPTECs associated with β2-microglobulin and preserved pH-dependent binding with IgG. In a steady state, FcRn in RPTECs was distributed on the cell surface and in the cytoplasm, indicating that FcRn-IgG interaction in RPTECs occurs both on the cell surface and in endocytic vesicles in agreement with recent studies (20, 24, 29). Indeed, human IgG could bind to the cell surface of the RPTECs in a pH-dependent manner.
with a high affinity at acidic pH and a low or no affinity at neutral pH. The preferential binding of IgG to RPTECs observed at pH 6.0 is consistent with the presence of FcRn on the cell surface. Furthermore, IgG was transported across the RPTEC monolayers bidirectionally, which required endosomal acidification. On the other hand, typical Fcγ receptors such as CD 16 (FcγRIII), CD 32 (FcγRII) and CD 64 (FcγRI) were not expressed in RPTECs at the mRNA level detected by RT-PCR. It was suggested that transepithelial transport of human IgG in RPTECs is mediated by FcRn. Co-localization of FcRn and the internalized human IgG shown by double immunofluorescence also supports the transepithelial transport of IgG in RPTECs by FcRn.

FcRn has been identified and characterized in various organs such as the small intestine, liver, mammary gland, placenta and yolk sac (17, 18, 29). This receptor is functionally expressed beyond the neonatal periods and is potentially relevant to other postnatal functions including the protection of IgG from catabolism (21). In all studies to date, multiple functions have been identified, but the molecular details of functions in distinct cellular environments are not fully understood (21). It is suggested that there are two important functions of this receptor. First, FcRn transports maternal IgG to the fetus or neonate. In rodents, maternal IgG transport occurs in the yolk sac or neonatal intestine, whereas in humans, essentially all IgGs are transferred prenatally across the placenta (17, 18). Second, FcRn is responsible for the maintenance of serum IgG levels by protecting plasma IgG from catabolism (22). These functions are supported by the fact that IgGs are salvaged from lysosomal degradation when they bind to FcRn, while IgG that does not bind to FcRn is destined for degradation in lysosomes (21). Since IgG is one of the most quantitatively important plasma proteins in the urine (2, 35) and accounts for most urinary immunoglobulins, we propose that IgG transport from the
apical to basolateral surface via FcRn in RPTECs reveals reabsorption of IgG from tubular fluid and that it may play an important role in IgG homeostasis (22).

Abbate et al. reported that interstitial cellular infiltration developed at or near tubules containing intracellular IgG or luminal casts under high levels of urinary protein excretion observed in different models of proteinuric nephropathies (4). In cultured proximal tubular cells, IgG stimulates synthesis of endothelin-1 and RANTES production, which may play a role in the interstitial inflammatory reaction (7, 36). As discussed above, excess reabsorption of IgG may be an important factor in the development and progression of chronic renal diseases.

IgG transport from the basolateral to apical surface via FcRn in RPTECs suggested that the proximal tubular epithelial cells may physiologically transport IgG to the mucosal surface. A previous study revealed that FcRn has important effects on IgG-mediated mucosal immunity and host defense in adult intestines using a human intestinal epithelial cell line (29). It has also been demonstrated that IgG is present in secretions of the human mucous membranes such as oral mucosa, lung, intestine and genitourinary tract (10, 29, 37-38). In the kidney, mucosal immune response has been intensively studied in urinary tract infections (11). For instance, urine from patients with urinary tract infections often contains antibodies against the infecting strain, particularly of secretory IgA (10). Polymeric immunoglobulin receptor which is produced by secretory epithelial cells transports polymeric IgA from the basolateral to the apical surface suggesting an important role in mucosal immunity of the urinary tract by secretion of secretory IgA (8, 9). Luminal secreted IgG may be of local origin and transported selectively across mucosal barriers in the same way as IgA (37,38).

In summary, we demonstrated that IgG is transported across RPTEC monolayers by
FcRn which requires endosomal acidification in binding IgG and prevents lysosomal degradation of IgG. Our studies not only identified receptor-mediated IgG transport in RPTECs but also raised the possibility that FcRn may have a role in the reabsorption of IgG from tubular fluid and in mucosal immunity of the urinary tract because of the bidirectional IgG transport in RPTECs. Further investigation is required to determine whether this receptor has functional relevance to these hypotheses in vivo.
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References


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Figure Legends

Fig. 1  hFcRn expression and physical association with β2- microglobulin in RPTECs

(A) RT-PCR analysis of T84 (lanes 1 and 2) and RPTECs (lanes 3 and 4). PCR was performed with primers specific for hFcRn cDNA and the products were analyzed on ethidium bromide-stained 2.0% agarose gel. Lanes 2 and 4, negative control (without reverse transcriptase); Lane 5, negative control (water as template); lane M, molecular base marker (100bp-ladder). (B) Immunoblot of cell extracts from RPTECs (lanes 1 and 4), Jurkat (lanes 2 and 5) and U937-hFcRn (lanes 3 and 6), probed with a rabbit anti-hFcRn antibody (lanes 1, 2 and 3) or pre-immune serum (lanes 4, 5 and 6). Total protein was loaded at 20 µg per lane. (C) Immunoprecipitates with rabbit anti-hFcRn antibody (lanes 1, 2 and 3) or pre-immune serum (lanes 5, 6 and 7). The proteins transferred onto the PVDF membrane were probed with a goat anti-human β2- microglobulin antibody. Lanes 1 and 5, U937-hFcRn; lanes 2 and 6, Jurkat; lanes 3 and 7, RPTECs; lanes 4 and 8, 0.3ng human β2- microglobulin. (D) Western blotting analysis of the cell lysates from RPTECs incubated with human IgG-agarose either at pH 6.0 or at pH 8.0. The membrane was probed with a rabbit anti-hFcRn antibody (lanes 1 and 2) or re-probed with a goat anti-human β2- microglobulin antibody (lanes 3 and 4).
**Fig. 2**  **Cell surface expression and steady state distribution of hFcRn in RPTECs**

RPTECs grown on the glass coverslips were fixed, either permeabilized (B and D) or not (A and C), and stained with a rabbit anti-hFcRn antibody followed by a rhodamine-labeled secondary antibody. Expression of hFcRn was detected at the cell surface (A) and the cytoplasm (B). No labeling was observed when RPTECs were stained with pre-immune serum and second antibody (C and D).

**Fig. 3**  **Gene expression of FcγRI (CD 64), FcγRII (CD 32) and FcγRIII (CD 16)**

Total RNA from the PBMC (lanes 1 and 2; positive control), U937 cells (lanes 6 and 7; positive control), and RPTECs (lanes 3, 4, 8 and 9) were incubated with an oligo(dT) primer with (lanes 1, 3, 6 and 8) or without (lanes 2, 4, 7 and 9) reverse transcriptase. PCR was performed with primers specific for each FcγR sequence or for GAPDH. PCR products were analyzed on an ethidium bromide-stained 2.0% agarose gel. Lanes 5 and 10, PCR negative control (water as template); lane M, molecular base marker (100bp-ladder).

**Fig. 4**  **pH-dependent binding of IgG to the cell surface of RPTECs**

Percent inhibition of biotinylated IgG binding at pH 6.0 and pH 8.0. Triplicate assays were performed in the absence (−, filled columns) or presence (+, open columns) of a 100-fold excess of unlabeled IgG, to assess the specificity of the binding. Typical result of Western blotting analysis as a heavy chain was shown. Data (mean ± SD) are expressed as % of binding in the absence of unlabeled IgG at pH 6.0.
Fig. 5  Receptor-mediated transcytosis of IgG across RPTECs monolayers

Transcytosis of biotinylated human IgG was detected in RPTECs monolayers at 37°C. Bidirectional transcytosis was significantly reduced in the monolayers pre-treated with 0.1 µM bafilomycin A1 and in the presence of a 100-fold excess of unlabeled human IgG but not chicken IgY. Apical to basolateral transport (A) and basolateral to apical transport (B) were shown. Data are expressed the mean ± SD of four different experiments.

Fig. 6  Co-localization of hFcRn and internalized IgG in RPTECs

RPTECs grown on glass coverslips were incubated at 4°C with human IgG in pH 6.0 or pH 8.0 solution. After washing and following incubation at 37°C, the internalized human IgG was detected with a FITC-conjugated goat anti-human IgG antibody (pH6.0; A, pH8.0; D). hFcRn was detected with a rabbit anti-hFcRn antibody (pH6.0; B, pH8.0; E). Merging A and B shows co-localization of internalized human IgG and hFcRn (C). F; Merging D and E, co-localization of human IgG (D) and hFcRn (E) were not observed.
Fig. 1

A

M  1  2  3  4  5

B

1  2  3  4  5  6

RPTECs  Jurkat  U937-hFcRn  RPTECs  Jurkat  U937-hFcRn

C

1  2  3  4

U937-hFcRn  Jurkat  RPTECs  β2-MG

14 kDa

1  2  3  4  5  6

14 kDa  14 kDa

1  2  3  4

pH 6.0  pH 8.0  pH 6.0  pH 8.0
Fig. 3
Fig. 4
Fig. 5

A

B

- untreated
- bafilomycin pre-treated
- IgG block
- IgY block

Apical to basolateral

Basolateral to apical
Fig. 6