Activation of Cyclosporin A Transport by a Novel λ Light Chain of Human Ig Surface Antigen-Related Gene in Xenopus laevis Oocytes

Yasuna Kobayashi, Takahiro Umemoto,1 Masayuki Ohbayashi, Noriko Kohyama, Yutaka Sanada, and Toshinori Yamamoto

Department of Clinical Pharmacy, School of Pharmacy, Showa University, Tokyo, Japan (Y.K., M.O., N.K., T.Y.); and Department of Surgery, School of Medicine, Showa University Fujigaoka Hospital, Kanagawa, Japan (T.U., Y.S.)

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ABSTRACT:

In the present study, we isolated and determined the pharmacological characteristics of a novel gene encoding the λ light chain of human Ig surface antigen-related gene (IgLC-rG). The isolated cDNA consisted of 693 base pairs that encoded a 232-amino acid protein. Northern blot analysis revealed that the IgLC-rG mRNA is expressed in the adult spleen and small intestine but not in fetal tissues. When expressed in Xenopus laevis oocytes, IgLC-rG mediated the high-affinity transport of [3H]cyclosporin A (CsA) (K_m = 189.7 ± 123.5 nM) in a sodium-dependent manner; however, other organic solutes such as p-aminophippuric acid and TEA were not transported via IgLC-rG. The transport of [3H]CsA by IgLC-rG was sensitive to pH. The uptake of [3H]CsA was trans-stimulated by CsA and GSH. Immunohistochemical analysis revealed that the IgLC-rG protein is localized at the brush border membrane in the human small intestine. Although the isolated IgLC-rG gene is a member of the human Ig λ light chain surface antigen superfamily, our findings suggest that IgLC-rG functions as a novel transport peptide responsible for CsA in the human body. Our results should provide insight into the novel function of membrane-bound proteins, such as Igls.

Introduction

The class of Igs is defined by the type of CH region. Igs are divided into five distinct isotypes: IgM, IgD, IgA, and IgE. Among them, IgM is the first Ig to be produced by any B cells, whereas IgG is the most abundant Ig that possesses the central ability to activate a complement. IgA is mainly produced by the tonsils and found in saliva. On the other hand, IgE is well known to be associated with allergic responses and defense against many kinds of allergens such as ticks, animal hairs, and exogenous drugs and chemicals. In general, these cell surface antigens are abundant Ig that possesses the central ability to activate a complement.

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1 Current affiliation: Department of Emergency and Critical Care Medicine, School of Medicine, Showa University Hospital, Tokyo, Japan.

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ABBREVIATIONS: OAT/Oat, organic anion transporter; SLC/Stc, solute carrier; OATP/Oatp, organic anion transporting polypeptide; TMDs, transmembrane domains; ABC, ATP-binding cassette; 4F2hc, 4F2 heavy chain; CsA, cyclosporin A; IgLC-rG, the λ light chain of human Ig surface antigen-related gene; PAH, p-aminophippuric acid; 6-MP, 6-mercaptopurine; MTX, methotrexate; TEA, tetraethylammonium; DEX, dexamethasone; dCTP, deoxycytidine [5'-α-32P]triphosphate; EST, expressed sequence tag; EMBL, European Molecular Biology Laboratory; DDBJ, DNA Data Bank of Japan; P0R, polymerase chain reaction; SSC, standard saline citrate; MES, 4-morpholineethanesulfonic acid; SASP, salazosulfapyridine, sulfasalazine; DMSO, dimethyl sulfoxide; kb, kilobase; VC, vitamin C; Ost, organic solute transporter; PG, prostaglandin; FK506, tacrolimus.
proteins actively transport various kinds of drugs and chemicals, assuming that, at least in part, transmembrane signature(s) are a structural requirement for stimulating the transport of organic solutes. CsA is a noncytotoxic immunosuppressant reagent and has been used for prophylaxis in organ transplantation. CsA has also been known to be a substrate of an ATP-dependent efflux transporter P-glycoprotein (Saeki et al., 1993). In addition, CsA is involved in drug-drug interactions (Sparrboom and Nooter, 2000; Xia et al., 2007), suggesting that there is some carrier protein responsible for CsA uptake; however, data concerning the uptake transporter for CsA are still limited.

In the present report, therefore, we describe the molecular cloning and functional characterization of a novel gene that belongs to the \( \lambda \) light chain of the human Ig surface antigen superfamily. We were surprised to find that an isolated gene, designated the \( \lambda \) light chain of human Ig surface antigen-related gene (IgLC-rG), stimulates the transport of CsA. Our results provide the first evidence of the transport of CsA via an Ig-related gene and IgLC-rG, which may be involved in CsA handling in the human body.

### Materials and Methods

#### Materials

- [\(^{3}H\)]CsA (7.0 Ci/mmol) was purchased from GE Healthcare UK Ltd. (Little Chalfont, Buckinghamshire, UK). [\(^{3}C\)]PAH (40.6 mCi/mmol), [\(^{3}C\)]-mercaptoaprine (6-MP) (53 mCi/mmol), [\(^{3}H\)]Haloquinarin (1.2 Ci/mmol), [\(^{3}H\)]methotrexate (MTX) (21.6 Ci/mmol), and [\(^{3}H\)]Hydralaprole (55 Ci/mmol) were purchased from Moravek Biosciences (Brea, CA). [\(^{3}H\)]Theophylline (52 mCi/mmol), [\(^{3}H\)]CCTEA (55 mCi/mmol), and [\(^{3}H\)]-carminine hydrochloride (80 Ci/mmol) were purchased from ARC Inc. (St. Louis, MO). [\(^{3}H\)]Dexamethasone (DEX) (35 Ci/mmol) was purchased from GE Healthcare UK Ltd. [\(^{14}C\)]-Ascorbic acid (vitamin C) (4 mCi/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). Deoxycytidine [\(^{5}\text{-}[\alpha-\text{32P}]\text{triphosphate (dCTP)}\) (111 TBq/mmol) was obtained from Murumachi Yakuhi Kaisha, Ltd. (Tokyo, Japan). All other chemicals not listed here were of the highest grade commercially available.

#### Construction of Human Small Intestine cDNA Library and Molecular Cloning of IgLC-rG

A nondirectional cDNA library was constructed from human small intestine poly(A)\(^{+}\) RNA using the Superscript Choice System (Invitrogen, Carlsbad, CA). The constructed cDNA library was ligated into a phage vector AZipLox EcoRI arms (Invitrogen). An expressed sequence tag (EST) clone (GenBank/EMBL/DDBJ accession number BX327815) was identified and amplified by PCR and labeled with [\(^{3}P\)]dCTP by random priming (\(^{3}P\)Quick Prime Kit; GE Healthcare). The library was screened with an EST clone as a probe under low stringency conditions. Replicate filters of a phage library were hybridized overnight in a hybridization solution (50% formamide, 5 mM NaCl, 2 mM KCl, 1 mM MgCl\(_2\), and 5 mM HEPES, pH 7.5) for at least 1 h at room temperature. The isolated clone, IgLC-rG, was linearized with KpnI (BspMII), and capped cDNA was transcribed in vitro by Sp6 RNA polymerase. Defolliculated oocytes were microinjected with 50 ng of in vitro transcribed cRNA and incubated for 2 to 3 days in a modified Barth’s solution containing gentamicin (50 \(\mu\)g/ml) at 18°C. Uptake experiments were performed in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 5 mM HEPES, pH 7.4) at room temperature. For the Na\(^{+}\) dependent or -independent uptake experiments, we used uptake buffers consisting of 96 mM NaCl, 96 mM LiCl, 96 mM choline chloride, 96 mM N-methyl-D-glucamine, or 96 mM mannitol. Oocytes were incubated in 150 \(\mu\)l of the same solution containing radiolabeled substrate for 1 h at room temperature in a 48-well plate, and the uptake was terminated by the addition of ice-cold ND96 solution. The oocytes were washed with the same solution at least five times after transfer to a 24-well plate. The oocytes were solubilized with 10% SDS, and accumulated radioactivity was determined with a liquid scintillation counter. The experiments were repeated with oocytes from at least three to five frogs.

#### Kinetic Study

Concentration-dependent uptake experiments of [\(^{3}H\)]CsA in oocytes expressing IgLC-rG were performed at final concentrations of 1, 50, 100, 200, and 600 nM. [\(^{3}H\)]CsA was incubated with oocytes expressing IgLC-rG for 1 h at room temperature, stopped with ice-cold ND96 solution, and washed 5 times as described above. Three oocytes were transferred to one scintillation vial and dissolved in 0.2 ml of 10% SDS. A scintillation cocktail was added, and radioactivity was counted. Counts in the control uninjected oocytes were subtracted from the counts in cRNA-injected oocytes. Data are presented as mean \(\pm\) S.E.M., except for kinetic constants for which the error represents the error of the fit. \(K_{m}\) indicates the Michaelis-Menten constant (nanomolar concentration).

#### cis-Inhibition Study

For inhibition experiments, oocytes expressing IgLC-rG were incubated for 1 h in ND96 solution containing 500 nM [\(^{3}H\)]CsA in the presence or absence of inhibitors at a final concentration of 50 \(\mu\)M. Digoxin, azithromycin, theophylline, captopril, and salazosulfapyridine (sulfasalazine, SASP) were directly dissolved in ND96 solution from the stock solution. These inhibitors were dissolved in dimethyl sulfoxide (DMSO) and diluted to a final concentration as described above. The final concentration of DMSO in the assay medium did not exceed 1%.

#### trans-Stimulatory Effect of CsA, Glutarate, and GSH on the Uptake of [\(^{3}H\)]CsA via IgLC-rG

The trans-stimulatory effect of CsA, glutarate, and GSH via IgLC-rG-mediated uptake of [\(^{3}H\)]CsA was examined under the following conditions: CsA, glutarate, and GSH were directly dissolved in ND96 solution from the stock solution; oocytes expressing IgLC-rG were preloaded with CsA, glutarate, and GSH at a final concentration of 50 \(\mu\)M and incubated for 1 h at room temperature; IgLC-rG-expressing oocytes were then incubated for 1 h in ND96 solution containing 500 nM [\(^{3}H\)]CsA. These stock solutions of the stimulator were dissolved in DMSO and diluted to a final concentration as described above. The final concentration of DMSO in the assay medium did not exceed 1%.

#### Immunohistochemical Analysis

A 5-\(\mu\)m wax section of human small intestine was obtained from BioChain Institute, Inc. (San Leandro, CA) and light microscopic immunohistochemical analysis using the streptavidin-biotin-horseradish peroxidase complex technique was carried out (Dako, Carpenteria, CA). Sections were dewaxed, rehydrated, and incubated with 3% H\(_2\)O\(_2\) in 100% methanol to eliminate endogenous peroxidase activity. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween 20, sections were treated with 200\(\times\) diluted (approximately 10 \(\mu\)g/ml) polyclonal antibody (amino acids 195–210, SSYLSLTPEQWKSHRS) at 4°C overnight. Thereafter, the sections were incubated with the secondary biotinylated goat polyclonal antibody (amino acids 195–210, SSYLSLTPEQWKSHRS) at 4°C overnight. Thereafter, the sections were incubated with the secondary biotinylated goat polyclonal antibody against rabbit Ig (Dako) with horseradish peroxidase-labeled streptavidin. This step was followed by incubation with diaminobenzidine and hydrogen peroxide. The sections were counterstained with Carazzi’s hematoxylin solution (Wako Pure Chemicals, Tokyo, Japan) and examined under light microscopy.
For the control experiment, the universal negative control rabbit solution (Dako) was used instead of IgLC-rG-specific antibody.

**Statistical Analysis.** Comparisons of data measuring the initial rates of uptake of \[^{[3]H}\]CsA in the presence and absence of inhibitors or stimulators were performed by the unpaired Student’s \( t \) test or two-way analysis of variance. Kinetic data from experiments measuring the uptake of radiolabeled substrates were fit to the Michaelis-Menten equation by nonlinear least-squares regression analysis with S.E.s derived from these curves.

**Results**

Using an EST clone (BX327815) as a probe, we screened a cDNA library under a low stringency condition (Umemoto et al., 2009) and isolated four positive clones designated the \( \lambda \) light chain of human Ig surface antigen-related gene, IgLC-rG. These clones had overlapping identical sequences and were 696 base pairs with a single open reading frame encoding a 232-amino acid sequence with a calculated molecular mass of 24.74 kDa (GenBank/EMBL/DDBJ accession number AB369252). There are no consensus sequences for \( O \)- and \( N \)-glycosylation sites, one consensus sequence for cAMP- and cGMP-dependent protein kinase C sites (Ser\(^{207}\)), and one consensus sequence for the cAMP phosphorylation site (Arg\(^{70}\)) (Fig. 1A). We found that there is an Ig domain variable region and an Ig domain constant region. In addition, the IgLC-rG amino acid sequence has one Marek’s disease glycoprotein A domain.

Kyte and Doolittle hydropathy analysis (window 9) revealed that IgLC-rG is likely to have two transmembrane domains. A similar result was obtained by TMpred analysis (http://www.ch.embnet.org/software/TMPRED_form.html), suggesting that IgLC-rG may have two transmembrane domains (TMD1 and TMD2) (Fig. 1B).

When we submitted the amino acid sequence of IgLC-rG to the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=...), we found the following alignment:

**Fig. 1.** A, cDNA and deduced amino acid sequences of the \( \lambda \) light chain of human Ig surface antigen-related gene, IgLC-rG. B, Kyte and Doolittle hydropathy plots (window 9) of IgLC-rG. C, phylogenetic relationship between IgLC-rG and several Ig light chain. The phylogenetic tree was constructed using DNASIS-Pro. Branch length is drawn to scale.
To elucidate the transport characteristics of IgLC-rG, we subsequently examined the effect of sodium-, pH-, time-, and concentration-dependent uptake. As shown in Fig. 4A, the uptake of [3H]Csa via IgLC-rG was affected by the replacement of extracellular sodium with choline, lithium, and mannitol. These results indicate that IgLC-rG is sodium-dependent. Pajor (1995) has reported that sodium dicarboxylate cotransporter-1 is a sodium-dependent transporter. Based on this report, we next examined sodium activation of [3H]Csa uptake in oocytes injected with IgLC-rG cRNA. As shown in Fig. 4B, when sodium was removed from the transport buffer and replaced with an equal amount of mannitol, the uptake of [3H]Csa was reduced to control levels. However, when sodium was restored in the transport buffer, trans-
port activity was recovered, indicating that IgLC-rG is a sodium-dependent carrier protein. The result was in good agreement with the result of Fig. 4A. The effect of pH on the transport of [3H]CsA by IgLC-rG-expressing oocytes was subsequently examined (Fig. 4C). There was a marked change in [3H]CsA transport with pH, with a much higher transport of [3H]CsA at an acidic pH and lower transport of [3H]CsA at an alkaline pH. Thus, the uptake of [3H]CsA mediated by IgLC-rG is sensitive to pH. The concentration dependence of the uptake of [3H]CsA by IgLC-rG is shown in Fig. 4D. Oocytes expressing IgLC-rG-mediated uptake of [3H]CsA showed saturable kinetics and could be modeled by the Michaelis-Menten equation. Nonlinear regression analyses yielded $K_m$ values of 189.7 ± 123.5 nM for CsA uptake, leading us to conclude that IgLC-rG is a sodium- and pH-dependent CsA transporter. IgLC-rG-mediated efflux of [3H]CsA was subsequently examined (Fig. 4E). As illustrated in Fig. 4E, the efflux of [3H]CsA via IgLC-rG was shown in a time-dependent manner up to 90 min. The IgLC-rG-mediated efflux of [3H]CsA reached a maximum at approximately 60 min; however, compared with the rates between influx and efflux, the influx rate was much higher (approximately 500 times higher) (Fig. 4A) than the efflux rate, indicating that IgLC-rG does not function as a bidirectional transporter.

**FIG. 3.** Uptake of various [H- or 14C-labeled compounds by IgLC-rG-expressing oocytes. The uptake rates of radiolabeled compounds (100 nM [3H]CsA, 100 nM [3H]DEX, 10 μM [14C]6-MP, 50 μM [14C]VC, 5 μM [14C]theophylline, 100 nM [3H]MTX, 100 nM [3H]valproate, and 2 μM [3H]allopurinol) by the control or IgLC-rG-expressing oocytes were measured for 1 h. Values are expressed as the mean ± S.E.M. of 12 to 18 oocyte determinations. The significance between control (water-injected) and IgLC-rG-cRNA-injected oocytes was determined by an unpaired $t$ test ($*$, $p < 0.01$). Other experimental conditions and details are described under Materials and Methods.
Li et al. (2000) have reported that intracellular GSH is an exchange substrate and GSH stimulates the transport of organic anions via Oatp2 [Oatp1a4 (Slc01a4)]. We have reported that transport of [3H]5-fluorouracil via the human nucleoside transporter 1 is not trans-stimulated by GSH and dicarboxylates (Umemoto et al., 2009). On the other hand, Mahagita et al. (2007) have reported that GSH has no effect on the uptake or efflux via OATP1B1/OATP-C (SLCO1B1) and/or OATP1B3/OATP-8 (SLCO1B8). Based on their findings, we next examined the trans-stimulatory effect of GSH and glutarate to elucidate whether these endogenous compounds trans-stimulate the transport of [3H]CsA via IgLC-rG. As shown in Fig. 5A, transport of [3H]CsA by IgLC-rG exhibited the trans-stimulatory effect in the presence of GSH; however, the uptake of [3H]CsA via IgLC-rG was not stimulated by preloading with glutarate. These results indicate that the influx of [3H]CsA mediated by IgLC-rG was significantly stimulated in the presence of CsA and GSH.

To define the substrate specificity of IgLC-rG further, the cis-inhibitory effect of various compounds by IgLC-rG-mediated uptake of [3H]CsA was subsequently investigated. As shown in Fig. 5B, we did not observe the cis-inhibitory effects for structurally unrelated compounds. Taken together, these results indicate that IgLC-rG is a sodium-, pH-, and concentration-dependent CsA transporter.

Immunohistochemical analysis was performed to determine the membrane localization of IgLC-rG in the human small intestine. As shown in Fig. 6A, low-power magnification light microscopy of 5-μm-thick paraffin-embedded sections demonstrated that there was immunostaining of IgLC-rG in the human small intestine. Under high magnification, IgLC-rG was located on the plasma membrane (Fig. 6B). By preincubation of normal rabbit serum, the immunoreactivity was diminished (data not shown).

Discussion

The present study describes the isolation and transport characterization of a novel gene encoding the light chain of the human Ig surface antigen-related gene, IgLC-rG. Transport experiments using a Xenopus oocyte expression system revealed that IgLC-rG predominantly transports [3H]CsA in pH-, concentration-, time-, and sodium-dependent manners. The uptake of [3H]CsA via IgLC-rG was trans-stimulated by GSH and CsA. Although IgLC-rG belongs to the Ig superfamily, the present article is the first evidence concerning the transport of organic solutes via the Ig superfamily-related gene in the human body.

The tissue distribution and the expression of the IgLC-rG gene were first investigated. The expression of IgLC-rG mRNA was predominantly expressed in the spleen and small intestine in human adult tissues, whereas expression in other tissues of the human body was low or none; therefore, we chose some human tissues (brain, liver, kidney, and placenta) and performed reverse transcription-PCR analysis to confirm whether the IgLC-rG gene is expressed. Although a faint PCR product was detected in the liver and kidney, no PCR product was found in the brain and placenta (data not shown), indicating that the expression of IgLC-rG mRNA is tissue-specific.

During the early period after birth, the gastrointestinal tract (e.g., small intestine) undergoes a complex process of growth and differentiation. Jenkins et al., (2003) have found that various enzymes such as lactase-phlorizin hydrolase (lactase), Fc receptor of the neonate...
mucosal adaptive immune systems also undergo dramatic changes during early postnatal development (Allan and Gleeson, 1999). Although we have not determined the precise mechanism of the ontogenic differences between adults and the fetus concerning IgLC-rG gene expression in the small intestine, several studies have suggested that other external stimuli, such as food intake, may also influence the precise timing and the level of expression of specific intestinal immune systems and protein development. Therefore, environmental changes and local stimuli functioning together may be responsible for initiating the expression of IgLC-rG gene in the small intestine. We also observed that IgLC-rG mRNA is not expressed in tumor-derived cell lines, suggesting that IgLC-rG may not be involved in the high protein synthesis for activation or growth in cancer cells.

As mentioned above, transporters such as OATP/Oatp (SLC21A/Slco21a), OAT/Oat (SLCO22A/Slco22a), and 4F2hc (SLC3A2), related to b0,+ amino acid transporter (D2) (SLC3A2), and Niemann-Pick C1-like 1 have several membrane-spanning domains, and these transporters, specifically the OAT and the OATP families, mediate a wide range of organic drugs and chemicals (Bertran et al., 1992a,b; Lee et al., 1993; Hagenbuch and Meier, 2003; Koepsell and Endou, 2004; Iyer et al., 2005). One possible structural signature for producing the transport activity seems to require the membrane-spanning domain(s). Therefore, we examined the secondary model of IgLC-rG using a Kyte and Doolittle hydrophathy analysis and found that IgLC-rG was predicted to have two membrane-spanning domains. On the basis of this finding together with published findings, we assumed that the isolated cDNA clone may have a transport function; therefore, IgLC-rG-cRNA-injected oocytes were used for transport characterization. We were surprised to find that IgLC-rG mediates the high-affinity transport of [3H]CsA in Na+-, pH-, time-, and concentration-dependent manners. Furthermore, we observed that oocytes expressing IgLC-rG do not transport PAH, TEA, and [scap]l-carnitine, suggesting that either anionic or cationic moieties would not be necessary for the transport recognition of IgLC-rG.

To elucidate the substrate selectivity of IgLC-rG, based on these findings, uptake experiments were subsequently performed using several radiolabeled compounds. However, we observed that DEX, 6-MP, VC, theophylline, MTX, valproate, and allopurinol did not transport IgLC-rG; IgLC-rG, however, transported CsA. These find-
ings lead us to conclude that CsA is a predominant substrate of IgLC-rG and may be the molecule responsible for the transport of CsA in the human body. Wang et al. (2001) and Seward et al. (2003) have revealed that both organic solute transporter α (Ostα) and organic solute transporter β (Ostβ) transport prostaglandin E2 (PGE2) and taurocholate. Because steroids and eicosanoids are involved in many cellular functions, it would be interesting to elucidate whether some steroids (estrone-3-sulfate, dehydroepiandrosterone sulfate, and taurocholate) and eicosanoids (PGE2 and PGF2α) are transported by IgLC-rG.

Several endogenous compounds have been identified as the driving force of drug transporters. Sekine et al. (1997) and Sweet et al. (1997) have reported that dicarboxylate is an endogenous substrate of rat Oat1 (Slc22a6) indicating that rat Oat1 (Slc22a6) acts as an organic anion/dicarboxylate exchanger. Dicarboxylates also act as the driving force of sodium dicarboxylate cotransporter-1 (Slc13a2) as reported by Pajour (1995). In addition, GSH is a substrate of Oat1 (Slc22a1), resulting in the transport of several drugs and chemicals as an exchange mechanism (Li et al., 2000), and Bahn et al. (2008) have revealed that human OAT10 (SLC22A13)-mediated uptake of [14C]urate is trans-stimulated by glutathione. To elucidate the driving force of IgLC-rG, based on these findings, we tested CsA, glutarate, and GSH. We found that a trans-stimulatory effect was observed with the addition of GSH and CsA, indicating that both compounds function as the driving force of IgLC-rG-mediated uptake and may act as a CsA/GSH exchanger in the human body.

Two conserved heterodimer interface domains (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) can be found on the IgLC-rG amino acid sequence. However, the Oste-Ostβ complex produced the maximum velocity as a drug transporter (Dawson et al., 2005), indicating that both proteins are necessary to produce the transport activity. Taking these facts into consideration, it is not clear at this time whether the IgLC-rG protein functions as a heterodimer or hetero­ oligomer to form the active transport molecule. Although phylogenetic tree analysis indicated that IgLC-rG is a member of the Ig light chain superfamily and Ig consists of two units (light and heavy chains), it would be interesting to elucidate whether this protein is required for the heavy chain such as 4F2hc (CD98) for LAT1 (Kanai et al., 1998) or whether the transport of CsA via IgLC-rG is a single transport system. In addition, it would be interesting to know whether IgLC-rG is coupled to other unidentified proteins. In vitro translation experiments would resolve this subject, or additional experiments involving the expression of IgLC-rG in transformed cell lines would better define the transport system for this clone.

We subsequently studied the cis-inhibitory effect of IgLC-rG-mediated uptake by several organic anions to clarify further the precise substrate selectivity. The transport of [3H]CsA mediated by IgLC-rG did not interact with any compounds such as digoxin, azithromycin, theophylline, captopril, and SASP. Because some of these drugs are known to cross the plasma membrane via OATP4C1 [OATP-H (SLC21A20)] (Mikkaichi et al., 2004) or human OAT2 (SLC22A7) (Kobayashi et al., 2005), our results indicate that, at least partly, these drugs may not be candidates for the substrate of IgLC-rG. Several investigators have shown that drug transporters such as peptide transporter-1 (SLC15A1) (Liang et al., 1995), monocarboxylate transporter 1 (SLC16A1) (Kim et al., 1992), OATP1A2/B2 (SLCO1A2/B2) (Kulkar-Ubllick et al., 1995), organic cation transporter novel 1/2 (SLC22A4/22A5) (Tamai et al., 1997; Wu et al., 1998), OCT3 (SLC22A3) (Kekuda et al., 1998), and Niemann-Pick C1-like 1 (Narushima et al., 2008) are expressed in the apical membrane of the small intestine. However, none of these transporters transport CsA. In consideration of these articles and our uptake experiments, IgLC-rG may play an important role in the intestinal handling of CsA.

Many kinds of drugs interact with CsA. Tacrolimus (FK506) has been used as a potent immunosuppressive drug for treatment of graft rejection in organ transplant patients or for treatment of some autoimmune diseases such as arthritis and atopic dermatitis (http://www.info.pmda.go.jp/search/html/menu_toplu_base.html). It is known that tacrolimus is metabolized by CYP3A4 (Shiraga et al., 1994). On the other hand, CsA is a substrate of CYP3A4; therefore, with the concomitant administration of both drugs, some contraindications could be observed in the clinical setting. To elucidate the mechanism of the transporter-mediated interaction of CsA with tacrolimus, we tested the concentration-dependent trans-stimulatory effect of tacrolimus on the uptake of [3H]CsA (data not shown). However, no trans-stimulatory effect was observed even at the highest concentration (10 μM), suggesting that IgLC-rG is not involved in the CsA-tacrolimus drug interaction.

For a comprehensive understanding of the role of IgLC-rG in the human body, we used a paraffin-embedded human small intestine and performed immunohistochemical analysis to determine the membrane localization of IgLC-rG. We found that IgLC-rG was localized on the apical membrane site of the human small intestine, suggesting that IgLC-rG acts as the entry of CsA from gastrointestinal contents into the bloodstream.

In conclusion, we describe the cloning and pharmacological char­acterization of a novel human gene, IgLC-rG. Phylogenetic tree analysis revealed that the amino acid sequence of IgLC-rG is positioned within a member of the Ig light chain superfamily. Therefore, our results are expected to facilitate research on the discovery of the novel function of membrane-anchored protein in the human body. In addition, our results may lead us to a new strategy to maximize the therapeutic efficacy and to minimize toxicity when targeting the IgLC-rG gene or its gene product in patients with autoimmune diseases or for treatment of graft rejection in organ transplant patients before and after the administration of CsA.

References


Address correspondence to: Dr. Toshinori Yamamoto, Department of Clinical Pharmacy, School of Pharmacy, Showa University, 1-5-8 Hatonodai, Shinagawa-ku, Tokyo 142-8555. E-mail: yamagen@pharm.showa-u.ac.jp