ACTIVATION OF CYCLOSPORIN A TRANSPORT BY A NOVEL LAMBDA LIGHT CHAIN OF HUMAN IMMUNOGLOBULIN SURFACE ANTIGEN–RELATED GENE IN XENOPUS LAEVIS OOCYTES

Yasuna K obayashi, Takahiro Umemoto, Masayuki Ohbayashi, Noriko Ohyama, Yutaka Sanada and Toshinori Yamamoto

Department of Clinical Pharmacy (Y.K., M.O., N.K., T.Y), School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
Department of Surgery (T.U., Y.S.), School of Medicine, Showa University Fujigaoka Hospital, 1-30 Fujigaoka, Aoba-ku, Yokohama-shi, Kanagawa 227-8501, Japan
A running title: Stimulation of CsA transport via IgLC-rG

Address correspondence to: Toshinori Yamamoto, Ph. D. Department of Clinical Pharmacy, School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142- 8555. Tel: +81-3-3784-8220. Fax: +81-3-3784-3838. Email: yamagen@pharm.showa-u.ac.jp

Number of text pages: 42
Number of tables: 1
Number of figures: 6
Number of references: 34
Number of words in Abstract: 197
Number of words in Introduction: 578
Number of words in Discussion: 1580

Abbreviations: A BCA1, A TP-binding cassette, sub-family A, member 1; ABC B1, ATP-binding cassette, sub-family B, member 1; ABCG2, ATP-binding cassette, sub-family G, member 2; BBM, brush border membrane; BLM, basolateral membrane; CsA, cyclosporine A; CYP3A4, cytochrome P450 3A4; dCTP, deoxycytidine [5'α-32P]triphosphate; DEX, dexamethasone; 4F2hc, 4F2 heavy chain; GSH, glutathione; hO SCP1, human organic solute carrier protein 1; Ig, immunoglobulin; IgLC-rG, the lambda light chain of human immunoglobulin surface antigen-related gene; kb, kilobase(s); MCTs, monocarboxylate transporters; MTX, methotrexate; 6-MP, 6-mercaptopurine; OAT, organic anion transporter; O ATP/oatp, organic anion transporting polypeptide; OC+, organic cation; OCT, organic cation transporter; PAH, p-aminophenolic acid; PEPT1, peptide transporter 1; rBAT, related to b0,+ amino acid transporter; SLC/SLC, solute carrier; TEA, tetrathylammonium; TMDs, transmembrane domains.
ABSTRACT:

In the present study, we isolated and determined the pharmacological characteristics of a novel gene encoding the lambda light chain of human immunoglobulin 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 tissue. When expressed in Xenopus laevis oocytes, IgLC-rG mediated the high affinity transport of [$^3$H]cyclosporine A (CsA) ($K_m = 189.7 \pm 123.5$ nM) in a sodium-dependent manner; however, other organic solutes such as $p$-aminohippuric acid (PAH) and tetraethylammonium (TEA) were not transported via IgLC-rG. The transport of [$^3$H]CsA by IgLC-rG was sensitive to pH. The uptake of [$^3$H]CsA was trans-stimulated by CsA and glutathione (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 immunoglobulin lambda 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 immunoglobulins.
Introduction

The class of immunoglobulins (Ig) is defined by the type of $C_H$ region. Igs are divided into five distinct isotypes such as IgM, IgD, IgG, IgA and IgE. Among them, IgM is the first immunoglobulin to be produced by any B cells, whereas IgG is the most abundant immunoglobulin 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. Generally, these cell surface antigens are believed to be bound in the cellular plasma membrane.

It is well known that drug transporters play a pivotal role in the drug clearance of the liver, kidney, brain, placenta, and small intestine. Such transporters have been predicted to have several membrane spanning domains and known to be localized at the cellular surface (Hagenbuch and Meier, 2004; Sekine et al., 2006). For example, the organic anion transporters (OATs/SLC22A) and the organic anion transporting polypeptides (OATPs/oatps[SLC21/SLCO]) are predicted to be bound at the sinusoidal membrane of hepatocytes, apical membrane of ileocytes and basolateral membrane of kidney proximal tubular cells, respectively, with 10-12 transmembrane domains (TMDs) (Hagenbuch and Meier, 2003; Hagenbuch and Meier, 2004; Koepsell...
Likewise, the ATP-binding cassette (ABC) transporter family, such as multidrug resistance proteins (MRPs), P-glycoprotein (P-gp/MDR1[ABCB1]) and breast cancer resistance protein (BCRP[ABCG2]) is predicted to have 17, 12 and 6 TMDs, respectively (Leslie et al. 2005). In addition, human organic solute carrier protein 1 (hOSCP1) is predicted to have 3 TDMs (Kobayashi et al., 2005). The 4F2 cell surface antigen (CD98) is a heterodimetric protein composed of two subunits, an 80-kDa glycosylated heavy chain and a 40-kDa non-glycosylated light chain (Haynes et al., 1981; Hemler et al., 1982). The 4F2hc antigen has been identified as a cell surface antigen and transport of a system b\(^{0,+}\)-like amino acid (Lee et al., 1993). The secondary structure model of 4F2hc is predicted to have a single transmembrane domain and is a transporter of amino acids when expressed in X. oocytes (Wells et al., 1992; Kanai et al., 1998). Similarly, related to b\(^{0,+}\) amino acid transporter (rBAT) induces dibasic and neutral amino acids (Betran et al., 1992a; Betran et al., 1992b). These membrane-bound proteins actively transport various kinds of drug 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 non-cytotoxic immunosuppressant reagent and has been used for prophylaxis of organ transplantation. CsA has been also known to be a substrate of an ATP-dependent efflux transporter P-glycoprotein (P-gp) (Saeki et al., 1993).
addition, CsA is involved in drug-drug interactions (Sparreboom and Nooter, 2000; Cindy et al., 2007); suggesting that there is some carrier protein responsible for CsA uptake; however, there is still limited data concerning the uptake transporter for CsA.

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 human immunoglobulin surface antigen superfamily. Surprisingly, we found that an isolated gene, designated the lambda light chain of human immunoglobulin surface antigen-related gene (IgLC-rG), stimulates the transport of CsA. Our results provide the first evidence of the transport of CsA via 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 GE Healthcare UK Limited (Buckinghamshire, U.K.). $[^{14}C]PAH$ (40.6 mCi/mmol), $[^{14}C]6$-mercaptopurine (6-MP) (53 mCi/mmol), $[^3]H]allopurinol$ (1.2 Ci/mmol), $[^3]H]methotrexate$ (MTX) (21.6 Ci/mmol) and $[^3]H]valproate$ (55 Ci/mmol) were purchased from Moravek (Brea, CA, U.S.A.). $[^{14}C]Theophylline$ (52 mCi/mmol), $[^{14}C]TEA$ (55 mCi/mmol), and $[^3]H]L$-carnitine hydrochloride (80 Ci/mmol) were purchased from ARC Inc. (St. Louis, MO, U.S.A.). $[^3]H]Dexamethasone$ (DEX) (35 Ci/mmol) was purchased from Amersham Biosciences UK Limited (Buckinghamshire, UK). $[^{14}C]L$-Ascorbic acid (vitamin C) (4 mCi/mmol) was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, U.S.A.). Deoxycytidine [$5'\alpha^{32}P]$triphosphate (dCTP) (111 TBq/mmol) was obtained from Muromachi Yakuhin 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 non directional cDNA library was constructed from human small intestine poly (A)+ RNA using the Superscript Choice System (Life Technologies, Gaithersburg, MD, U.S.A.). Constructed cDNA library was ligated into a phage vector $\lambda$ZipLox EcoRI arms (Life Technologies, Inc., Gaithersburg, MD, U.S.A.). An expressed sequence tag (EST) clone (GenBank™/EMBL/DDBJ accession number:...
BX327815) was identified and amplified by PCR and labeled with $[^{32}\text{P}]$dCTP by random priming (T7Quick Prime Kit, Amersham Pharmacia Biotech). 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; 5x standard saline citrate (SSC); 3x Denhardt’s solution; 0.2% SDS; 10% dextran sulfate; 0.3 μg/ml denatured salmon sperm DNA; 2.5 M sodium pyrophosphate; 25 mM MES; 0.03% Antifoam A; pH 6.5) at 37°C overnight. The filters were washed in 3 × SSC and 0.5% SDS at 37°C. cDNA inserts in positive λZipLox phage were recovered in a plasmid pZL1 vector by in vitro excision.

DNA Sequence and Hydropathy Analysis. Double-stranded cDNA of isolated clones obtained from the library were sequenced in both directions. Using a dye primer cycle sequencing kit (ver. 3.1, Applied Biosystems, Foster City, CA, U.S.A.) and automated Applied Biosystems 310 DNA sequencer, deleted clones were made using a KiloSeq uence deletion kit (Takara, Tokyo, Japan). The sequence, membrane topology, and the presence of possible signal peptides were assembled and analyzed using DNASIS-Pro. Version 2.02 (Hitachi Software Engineering, Kanagawa, Japan).

Northern Blotting Hybridization. A commercially available hybridization blot containing poly (A)$^+$ RNA from various human tissues (Human M TN Blot I and II,
CLONTECH, (Palo Alto, CA, USA) and human cancer cell line MTN blots (CLONTECH, Palo Alto, CA, USA) were used for the Northern blot analysis. These filters were hybridized at 42°C overnight in a hybridization solution (50% formamide) with full-length cDNA of IgLC-rG, which was randomly labeled with $^{32}$P-dCTP as described above. The membrane was washed in 0.1x SSC/0.1% SDS at 42°C.

**Preparation of IgLC-rG cRNA and Transport Measurement.** Stages V and VI defolliculated Xenopus oocytes were isolated from the female frog and used throughout this study. X. oocytes were treated with collagenase A (Boehringer Mannheim, Mannheim, Germany, 2.0 mg/ml) in a oocyte Ringer 2 (OR2) solution (83 mM NaCl, 2 mM KCl, 1 mM MgCl$_2$, 5 mM HEPES, pH 7.5) for at least 1 h at room temperature. The isolated clone, IgLC-rG, was linearized with Kpn2I (BspMII), and capped complementary RNA (cRNA) was transcribed *in vitro* by Sp6 RNA polymerase. Defolliculated oocytes were microinjected with 50 ng of *in vitro* transcribed cRNA and incubated for two to three days in a modified Barth’s solution containing gentamicin (50 μg/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$_2$, 5 mM HEPES, pH 7.4) at room temperature. For the Na$^+$-dependent or -independent uptake experiments, we employed uptake buffers consisting of 96 mM NaCl, 96 mM LiCl, 96 mM choline...
chloride, 96 mM N-methyl-D-glucamine (NMDG) or 96 mM mannitol. Oocytes were incubated in 150 μl of the same solution containing radiolabeled substrate for 1 hr 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 [3H]CsA in oocytes expressing IgLC-rG were performed at final concentrations of 1, 50, 100, 200, and 600 nM. [3H]CsA was incubated with oocyte 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 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 ± S.E.M., except for kinetic constants for which the error represents the error of the fit. \( K_m \) indicates the Michaelis-Menten constant (nM).

**cis-Inhibition Study.** For inhibition experiments, oocytes expressing IgLC-rG were incubated for 1 h in ND96 solution containing 500 nM [3H]CsA in the presence or
absence of inhibitors at a final concentration of 50 μM. Digoxin, azithromycin, theophylline, captopril and alazosulfapyridine (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 Glutathione (GSH) on the Uptake of [3H]CsA via IgLC-rG.** The *trans*-stimulatory effect of CsA, glutarate and glutathione (GSH) via IgLC-rG-mediated uptake of [3H]CsA was examined by the following conditions: CsA, glutarate, and GSH were directly dissolved in ND96 solution from the stock solution; oocyte expressing IgLC-rG was preloaded with CsA, glutarate, and GSH at a final concentration of 50 μM and incubated for 1 h at room temperature; IgLC-rG-expressing oocytes were then incubated for 1 h in ND96 solution containing 500 nM [3H]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 five-micrometer wax section of human small intestine was obtained from BioChain Institute, Inc. (San Leandro, CA, U.S.A.) and light microscopic immunohistochemical analysis using the streptavidine-biotin-horseradish peroxidase complex technique was carried out.
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 200x diluted (about 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 against rabbit immunoglobulin (DAKO, Carpinteria, CA, U.S.A.) 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, Tokyo, Japan) and examined under light microscopy. For the control experiment, the universal negative control rabbit solution (DAKO, Carpinteria, CA, U.S.A.) was used instead of IgLC-rG-specific antibody.

**Statistical Analysis.** Comparisons of data measuring initial rates of uptake of [$^3$H]CsA in the presence and absence of inhibitors or stimulators were performed by the un paired Student’s $t$ test or two-way ANOVA. Kinetic data from experiments measuring the uptake of radiolabeled substrates were fit to the Michaelis-Menten equation by nonlinear least-squares regression analysis with standard errors derived from these curves.
RESULTS

Using an EST clone (BX327815) as a probe, we screened a cDNA library under low stringency condition (Umemoto et al., 2009) and isolated four positive clones designated the lambda light chain of human immunoglobulin surface antigen-related gene, IgLC-rG. These clones had overlapping identical sequences and were 696 bp 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 (PKC) sites (Ser207), and one consensus sequence for the cAMP phosphorylation site (Arg70) (Fig. 1A). We found that there is an immunoglobulin domain variable (IGv) region and an immunoglobulin domain constant region (IGc). In addition, 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 (TMD1-2) (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=blastp&BLAST_PROGRAMS=bla
several similar sequences were identified. Therefore, we constructed the phylogenetic tree using some of these similar sequences the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and found that IgLC-rG is positioned between the human immunoglobulin lambda light chain and immunoglobulin light chain (Fig. 1C). The analysis of this protein sequence using the ScanProsite program (http://www.expasy.ch/) predicted that there are two Ig-like domain sequences (Pro5 to Ala108 and Pro133 to Ala227) and major histocompatibility complex (MHC) proteins signature (Tyr211 to His217). The nucleotide sequence of IgLC-rG is 95% identical to that of human Ig lambda chain (accession number S25753) except for 12 amino acids: (Leu28 to Ser, Lys45 to Thr, Asn49 to Lys, Leu56 to Gln, Gln60 to His, Tyr67 to Phe, Asn70 to Ser, Arg71 to Lys, Ile80 to Phe, Glu101 to Asp, Ser112 to Ala, Trp113 delete). These findings lead us to conclude that the isolated cDNA clone is a novel gene that belongs to the human Ig superfamily.

Tissue expression of the mRNA corresponding to IgLC-rG in human various tissues was subsequently examined by Northern blot analysis. As shown in Fig. 2A, two strong 0.7 kb mRNA bands were detected predominantly in the human adult spleen and small intestine. However, no significant 0.7 kb mRNA was detected in other adult tissues of the heart, brain (whole), placenta, lung, liver, skeletal muscle,
kidney, pancreas, thymus, prostate, testis, ovary, colon, prostate, peripheral blood leucocytes as well as in human fetal tissues (Fig. 2A). Thus, the expression of IgLC-rG mRNA in humans was tissue-specific. Based on these observations, we next examined the distribution of IgLC-rG mRNA in mouse tissues by Northern blot analysis in order to elucidate whether there exists a counterpart of this clone. The result is also shown in Fig. 2. However, no IgLC-rG mRNA was expressed in the male mouse tissues (data not shown). Moreover, we could not detect any bands corresponding to the IgLC-rG gene in all tissues of the guinea pig (data not shown), indicating that the IgLC-rG gene is human-specific. Further, we found that there was no signal detected in several human tumor-derived cell lines such as HL60, HeLa S3, MOLT-4, Raji, SW480, A549, and G361 (Fig. 2B).

Because the IgLC-rG amino acid sequence is predicted to have two membrane spanning domains (Fig. 1), we assumed that this clone can transport organic solutes. Therefore, we used a Xenopus laevis oocyte expression system and IgLC-rG cRNA-injected X. oocytes to determine the transport characterization. Since [14C]PAH, [14C]TEA, and [3H]L-carnitine were typical substrates for the OATs[SLC22A], the OC Ts[SLC22A], and the organic cation transporter novel (OCTNs[SLC22A]) families, respectively, we firstly tested these compounds to elucidate whether these compounds are transported by IgLC-rG. However, as shown in Table 1, no
significant transport activity was observed. To search further possible substrates of IgLC-rG, [3H]CsA, [3H]DEX, [14C]6-MP, [14C]ascorbic acid (vitamin C, VC), [14C]theophylline, [3H]MTX, [3H]valproate, and [3H]allopurinol were used for transport experiments. As illustrated in Fig. 3, none of these substrates were transported via IgLC-rG except [3H]CsA. The finding indicates that CsA is a predominant substrate of IgLC-rG; therefore, we employed [3H]CsA as a substrate for the further pharmacological characterization of IgLC-rG.

To elucidate the transport characteristics of IgLC-rG, we subsequently examined the effect of sodium-, pH-, time- and concentration-dependence 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 has reported that sodium dicarboxylate cotransporter-1 (NaDC-1) is a sodium-dependent transporter (Pajor, 1995). 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, transport 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 $[^3]$H]CsA by IgLC-rG-expressing oocytes was subsequently examined (Fig. 4C). There was a marked change in $[^3]$H]CsA transport with pH, with a much higher transport of $[^3]$H]CsA at an acidic pH and lower transport of $[^3]$H]CsA at an alkalic pH. Thus, the uptake of $[^3]$H]CsA mediated by IgLC-rG is sensitive to pH. The concentration dependence of the uptake of $[^3]$H]CsA by IgLC-rG is shown in Fig. 4D. Oocyte expressing IgLC-rG-mediated uptake of $[^3]$H]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 $[^3]$H]CsA was subsequently examined (Fig. 4E). As illustrated in Fig. 4E, the efflux of $[^3]$H]CsA via IgLC-rG was shown in a time-dependent manner up to 90 min. The IgLC-rG-mediated efflux of $[^3]$H]CsA reached a maximum at around 60 min; however, when compared with the rates between influx and efflux, the influx rate was much higher (approximately 500 times higher, Fig. 4A) than that of the efflux rate, indicating that IgLC-rG does not function as a bidirectional transporter.

Li et al. have reported that intracellular GSH is an exchange substrate and GSH stimulates the transport of organic anions via Oatp2 (Oatp1a4[S/col1a4]) (Li et al., 1998). We have recently reported that transport of $[^3]$H]5-FU via hNT1 is not
trans-stimulated by GSH and dicarboxylates (Umemoto et al., 2009). On the other hand, Mahagita et al. have recently reported that GSH has no effect on the uptake or efflux via OATP1B1/OATP-C[SLOC1B1] and/or OATP1B3/OATP-8[SLOC1B1] (Mahagita et al., 2007). 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 find further the substrate specificity of IgLC-rG, 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, 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 lambda light chain of human immunoglobulin surface antigen-related gene, IgLC-rG. Transport experiments using a X. oocyte expression system revealed that IgLC-rG predominantly transports $[^3]$H CsA in pH-, concentration-, time-, and sodium-dependent manners. The uptake of $[^3]$H CsA via IgLC-rG was trans-stimulated by GSH and CsA. Although IgLC-rG belongs to the Ig superfamily, the present paper 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 firstly 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 RT-PCR analysis in order 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 such as the small intestine undergoes a complex process of growth and differentiation. Jenkins et al.
have found that various enzymes such as lactase-phlorizin hydrolase (lactase), Fc receptor of the neonate (FcRn) and polymeric IgA receptors (pIgR), an apical sodium-dependent bile acid transporter (SLC10A2), and an Na+/glucose cotransporter (SLC5A1) are expressed in enterocytes. Except lactase, these proteins are gradually increased with each day after birth, and it is developmentally regulated by the postnatal development (Jenkins et al., 2003). Likewise, the 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 the 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 high protein synthesis for activation or growth in cancer cells.

As mentioned above, transporters such as OATP/Oatp[SLCO21A/Slco21a],
OAT/Oat[SLCO22A/Slco22a], 4 F2hc[SLC3A2], rBAT (D2) [SLC3A1], and Niemann-Pick C1-like 1 (NPC1L1) have several membrane-spanning domains, and these transporters specifically the OAT and the OATP families mediate a wide range of organic drugs and chemicals (Lee et al., 1993; Betran et al., 1992a; Betran et al., 1992b; 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 hydropathy 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. Surprisingly, we found that IgLC-rG mediates the high affinity transport of [³H]CsA in a Na⁺-, pH-, time-, and concentration-dependent manners. Further, we observed that oocytes expressing IgLC-rG do not transport PAH, TEA and 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 DX, 6-MP, VC, theophylline, MTX, valproate, and
allopurinol did not transport IgLC-rG; IgLC-rG, however, transported CsA. These findings lead us to conclude that CsA is a predominant substrate of IgLC-rG and may be the responsible molecule for the transport of CsA in the human body. Wang et al. and Seward et al. have revealed that both organic solute transporter α (Ostα) and organic solute transporter β (Ostβ) transport prostaglandin E₂ (PGE₂) and taurocholate (Wang et al., 2001; Seward et al., 2003). 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. and Sweet et al. have reported that dicarboxylates is an endogenous substrate of rat Oat1 (rOat1[Slc22a6]) indicating that rOat1[Slc22a6] acts as an organic anion/dicarboxylates exchanger (Sekine et al., 1997; Sweet et al., 1997). Dicarboxylate also acts as the driving force of sodium-dicarboxylate cotransporter-1 (NaDC-1[Slc13a2]) as reported by Pajor (Pajor, 1995). In addition, GSH is a substrate of Oatp1[Slco1a1] and resulting in the transport of several drugs and chemicals as an exchange mechanisms (Li et al., 1998). More recently, Bahn et al. have revealed that hOAT10[SLC22A13]-mediated uptake of [³¹⁴C]urate is trans-stimulated by glutathione (Bahn et al., 2008). To elucidate the driving force of
IgLC-rG, based on these findings, we tested CsA, glutarate and GSH as a driving force of IgLC-rG. We found that a trans-stimulatory effect was observed when adding 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 can be found on the IgLC-rG amino acid sequence. However, Ostα-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 immunoglobulin 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 in volving the expression of IgLC-rG in transformed cell lines would be better define the transport
system regarding 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. Since some of these drugs are known to cross the plasma membrane via OATP-H ([SLC21A20]) (Mikkaichi et al., 2004) or human OAT2 (hOAT2[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 elucidated that drug transporters such as peptide transporter-1 (PEPT1[SLC15A1]) (Liang et al., 1995), monocarboxylate transporter 1 (MCT1[SLC16A1]) (Kim et al., 1992), OATP1A2/2B1[SLCO1A2/2B1] (Kullak-Ublick et al., 1995), organic cation transporter novel 1/2 (OCTN1/2[SLC22A4/22A5]) (Tamai et al., 1997; Wu et al., 1998), organic cation transporter 3 (OCT3/SLC22A3) (Kekuda et al., 1998), NPC1L1 (Narushima et al., 2008) are known to be expressed in the apical membrane of the small intestine. However, none of these transporters transport CsA. Taking these papers and our uptake experiments into consideration, 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 transplantation patients, or for treatment of several autoimmune diseases such as arthritis and atopic dermatitis (http://www.info.pmda.go.jp/psearch/html/menu_tenpu_base.html). It is known that tacrolimus (FK506) is metabolized by cytochrome P450 3A4 (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 (FK506), we tested the concentration-dependent trans-stimulatory effect of tacrolimus (FK506) 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 (FK506) drug interaction.

For a comprehensive understanding of the role of IgLC-rG in the human body, we employed 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. Our results, therefore, 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 transplantation patients before and after the administration of CsA.
Acknowledgments

This work was supported in part by Grants-in-Aid for Exploratory Research (19659329) and "High-Tech Research Center Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) 2005-2009. The nucleotide sequence reported in this paper has been submitted to the GenBank™/DDBJ/EMBL Data Bank with accession number(s) AB369252.
REFERENCES


Footnote:

§Present address: Department of Emergency and Critical Care Medicine, School of Medicine, Showa University Hospital, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.
FIGURE LEGENDS

Fig. 1. A, cDNA and deduced amino acid sequences of the lambda light chain of human immunoglobulin surface antigen-related gene, IgLC-rG. B, Kyte and Doolittle hydropathy plot s (window 9) of IgLC-rG. C, Phylogenetic relationship between IgLC-rG and several immunoglobulin light chains. The phylogenetic tree was constructed using DNAsis-Pro. Version 2.02 (HITACHI Software Engineering). Branch length is drawn to scale. TDM, transmembrane domain; IgLC-rG, the lambda light chain of human immunoglobulin surface antigen-related gene.

Fig. 2. Localization and tissue distribution of IgLC-rG mRNA by Northern blot analysis. A, a high-stringency Northern blot analysis of poly(A)⁺ RNA from various tissues of human adults (CLONTECH) probed with ³²P-labeled IgLC-rG cDNA. A highly expressed 0.7 kb transcript was detected in the spleen and small intestine. In the fetal tissues, no transcript was detected in the brain, kidney, lung and small intestine. B, a high-stringency Northern blot analysis in human tumor-derived cells. No transcript was detected in all cell lines. Each panel shows a 2-day exposure. Other experimental conditions and details are described in Experimental Procedures. HL60, promyelocytic leukemia; HeLa S3, HeLa subcell line 3; K 562, chronic myelogenous leukemia; MOLT-4, lymphoblastic leukemia; Raji, Burkitt’s lymphoma;
SW480, colorectal adenocarcinoma; A549, lung carcinoma; G361, melanoma.

**Fig. 3.** Uptake of various $^3$H- or $^{14}$C-labeled compounds by IgLC-rG-expressing oocytes. The uptake rates of radiolabeled compounds ($[^3]$H]CsA, 100 nM; $[^3]$H]DEX, 100 nM; $[^14]$C]6-MP, 10 μM; $[^14]$C]VC, 50 μM; $[^14]$C]theophylline, 5 μM; $[^3]$H]MTX, 100 nM; $[^3]$H]valproate, 100 nM; $[^3]$H]allopurinol, 2 μM) by the control or IgLC-rG-expressed oocytes were measured for 1 h. Values are expressed the mean ± S.E.M. of 12 ~ 18-oocyte determinations. The significance between control (water-injected) and IgLC-rG-cRNA-injected oocytes was determined by the unpaired t test (*p<0.01). Other experimental conditions and details are described in Experimental Procedures. DEX, dexamethasone; 6-MP, 6-mercaptopurine; VC, vitamin C; MTX, methotrexate.

**Fig. 4.** Transport Characterization of IgLC-rG. A, effect of extracellular cation on $[^3]$H]CsA in *Xenopus laevis* oocytes expressing IgLC-rG. The uptake rates of $[^3]$H]CsA (500 nM) by control oocytes or IgLC-rG-expressing oocytes for 1 h, respectively, were measured in the presence or absence of extracellular Na$^+$. Extracellular Na$^+$ was replaced with an equimolar concentration of choline, lithium, mannitol, and N-methyl-D-glucamine (NMDG). B, sodium-dependent activation of $[^3]$H]CsA uptake in oocytes injected with IgLC-rG cRNA. One-hour uptake of 500 nM $[^3]$H]CsA was
measured in the presence of increasing concentrations of sodium in the uptake buffer (ND96). The NaCl was replaced by equimolar mannitol. Values are expressed the mean ± S.E.M. of 12–15-oocyte determinations. C, Effect of pH on [3H]CsA transports in IgLC-rG-expressing oocytes. One-hour uptake of 500 nM [3H]CsA was measured in Na+ buffers adjusted to pH values ranging from 5.5 to 8.5. D, Concentration-dependence of IgLC-rG-mediated uptake of [3H]CsA. The uptake rates of CsA by the control and IgLC-rG-expressing oocytes for 1 h were measured at variable concentrations (mean ± S.E.M., n = 12–15 oocytes). E, Time-dependent efflux of [3H]CsA by IgLC-rG-expressed oocytes. After one-hour uptake of 500 nM [3H]CsA in oocytes expressing IgLC-rG, oocytes were transferred to ND96 solution. The efflux amount of CsA during 90 min was shown as fmol/oocyte. Data are mean ± S.E.M. (n = 12–18 oocytes). F, Time course of CsA uptake by IgLC-rG-expressed oocytes. The uptake of 500 nM [3H]CsA in oocytes expressing IgLC-rG was measured during 2.0 h of incubation. Data are mean ± S.E.M. (n = 12–15 oocytes).

Other experimental conditions and details are described in Experimental Procedures. IgLC-rG, the lambda light chain of human immunoglobulin surface antigen-related gene.

**Fig. 5.** A, trans-Stimulatory effect of CsA, glutarate and GSH on the transport of
\[^3\text{H}\]CsA via IgLC-rG. 500 nM \[^3\text{H}\]CsA was used for the uptake experiments. Oocytes expressing IgLC-rG was preloaded with 50 μM cyclosporine A, 50 μM glutarate or 50 μM GS H for 1 h before starting the uptake experiment. \textit{trans}-Stimulatory effect was observed in the presence of CsA and GSH. Data are expressed as fmol/oocyte/h. Values are mean ± S.E.M. of 18 ~ 21-oocyte determinations. B, \textit{cis}-Inhibitory effect of IgLC-rG-mediated \[^3\text{H}\]CsA uptake by various compounds. The concentration of \[^3\text{H}\]CsA was 500 nM and those of inhibitors in the assay medium were 50 μM. The values are expressed as a percentage of IgLC-rG-mediated \[^3\text{H}\]CsA uptake in the absence of inhibitors. Data are expressed as the mean ± S. E.M. of 18–21 oocytes. Other experimental conditions and details are described in \textit{Experimental Procedures}. IgLC-rG, the lambda light chain of human immunoglobulin surface antigen-related gene; SASP, salazosulfapyridine (sulfasalazine).

\textbf{Fig. 6.} Immunohistochemical analysis of IgLC-rG in human small intestine. Five-micrometer sections were incubated with polyclonal anti-IgLC-rG antibody. The brush border membrane site of the small intestine was stained (A, x200; B, x400). Diagram of membrane localization of IgLC-rG in small intestine (C). Other experimental conditions and details are described in \textit{Experimental Procedures}. 
ABCA1, A TP-binding cassette, sub-family A, member 1; A BCB1, A TP-binding cassette, sub-family B, member 1; ABCG2, ATP-binding cassette, sub-family G, member 2; BBM, brush border membrane; BLM, basolateral membrane. CsA, cyclosporine A; GSH, glutathione; IgLC-rG, the lambda light chain of human immunoglobulin surface antigen-related gene; MCTs, monocarboxylate transporters; OATPs, organic anion transporting polypeptides; OC+, organic cation; OCT, organic cation transporter; PEPT1, peptide transporter 1.
Table 1. Uptake of $[^{14}C]p$-aminohippuric acid, $[^{14}C]$tetraethylammonium, and $[^{3}H]$L-carnitine by human immunoglobulin lambda light chain surface antigen-related gene-expressing oocytes. After two days incubation, uptake experiments were performed in a solution containing Na$^+$ for 1 h. Values are mean ± S.E.M. of 9-15 oocyte determinations. The units of measure were femtomoles per oocyte per hour for $[^{3}H]$L-carnitine uptake and picomoles per oocyte per hour for $[^{14}C]p$-aminohippuric acid and $[^{14}C]$tetraethylammonium uptake, respectively. The significance between control (non-injected) and IgLC-rG-cRNA-injected oocytes was determined by using one-way ANOVA ( *$p<0.05$). Other experimental conditions and methods are described under Experimental Procedures. PAH, $p$-aminohippuric acid; TEA, tetraethylammonium; IgLC-rG, the lambda light chain of human immunoglobulin surface antigen-related gene.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Concentration</th>
<th>Non-injected</th>
<th>IgLC-rG-cRNA injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[^{14}C]$PAH</td>
<td>10 μM</td>
<td>0.45 ± 0.19</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>$[^{14}C]$TEA</td>
<td>10 μM</td>
<td>0.11 ± 0.06</td>
<td>0.10 ± 0.07</td>
</tr>
<tr>
<td>$[^{3}H]$L-Carnitine</td>
<td>0 nM</td>
<td>41.20 ± 4.65</td>
<td>35.82 ± 5.09</td>
</tr>
</tbody>
</table>
Fig. 1.

A

B

C

DMD Fast Forward. Published on June 14, 2010 as DOI: 10.1124/dmd.109.030916
This article has not been copyedited and formatted. The final version may differ from this version.
Fig. 2.

A

<table>
<thead>
<tr>
<th>Human</th>
<th>Adult</th>
<th>Fetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>kb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.
Fig. 4.

A) Na⁺, Li⁺, Choline, Mannitol, NMDG

B) pH

C) Na⁺ Concentration (mM)

D) Concentration (nM)

E) Time (min)

F) Time (min)
**Fig. 5.**

(A) Bar graph showing the uptake of [3H]Cyclosporin A in oocytes treated with various compounds. The compounds include Control, IgLC-rG, Cyclosporin A, Glutarate, and Glutathione.

(B) Bar graph illustrating the percentage of Control uptake with the addition of different inhibitors. The inhibitors are Control, Digoxin, Azithromycin, Theophylline, Captopril, and SASP.