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CD300 antigen like family member G: A novel Ig receptor like protein exclusively expressed on capillary endothelium

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Abstract

We report the characteristics of CD300LG, a member of the CD300 antigen like family. Its genomic structure is similar in both mouse and human, and at least four isoforms exist in both species. The amino acid sequence of the immunoglobulin (Ig) V like domain of CD300LG showed approximately 35% identity to those of the polymeric Ig receptor (pIgR) and Fcα/μR. Interestingly, mouse CD300LG proteins were uniquely expressed on capillary endothelium. Immunoelectron microscopy revealed that mouse CD300LG is localized on both apical and basolateral plasma membranes, as well as on intracellular vesicular structures, in the capillary endothelium. Transcytosis assays using polarized MDCK epithelial cells showed that CD300LG could be transcytosed bidirectionally. Furthermore, CD300LG exogenously expressed on HeLa cells could take up IgA2 and IgM, but not IgG. These results suggest that CD300LG might play an important role in molecular traffic across the capillary endothelium.

Keywords: CD300L; CMRF35; Immunoglobulin super family; Capillary endothelium; Transcytosis; IgA; IgM

The Ig superfamily (IgSF) consists of a number of cell surface glycoproteins that share sequence homology with the V and C domains of antibody (Ab) heavy and light chains, and is involved in many important functions including leukocyte adhesion, antigen recognition, and activation and inhibition of immune response [1,2]. Currently, several members of the IgSF are grouped into smaller families that have related genes or, alternatively, several isoforms of different molecules such as killer cell Ig like receptors [3], leukocyte Ig like receptors [4], Fc receptors [5], signal-regulatory proteins [6], triggering receptor expressed on myeloid cells (TREM) [7], paired Ig like type 2 receptor [8], and CD300L [9].

The CD300L family is widely found on most hematopoietic cells including monocytes, neutrophils, macrophages, dendritic cells, subpopulations of lymphocytes and bone marrow cells [9]. Each member of this family is a type I cell surface glycoprotein containing a single Ig V like domain. In the past GenBank database, some mouse genes of the CD300L family had been termed polymeric Ig receptor (pIgR) precursors because their Ig V like domains have significant sequence homologies to that of pIgR, the Fc receptor for polymeric IgA and IgM [10]. On the other hand, human genes of the CD300L family have been identified as an antigen to the CMRF35 monoclonal antibody (mAb) [11]. Subsequently, it has been revealed that the human CMRF35 antigens are orthologs of mouse pIgR precursors. Recently, this family has been renamed CD300 antigen like (A–G) in both mouse and human. It has been demonstrated that the CD300L family

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also exists in rat and chicken [12]. Although the function of the CD300L family has remained elusive, their locus in human has been recently linked to psoriasis susceptibility [13], suggesting that the CD300L family might also act as important receptors in some inflammatory responses.

Although the extracellular region of the CD300L family has certain homology with the Ig domains of pIgR and Fcα/μR, it has been regarded, albeit lack of strong evidence, that the CD300L family could not bind Igs. In fact, the CD300L family remains poorly examined in terms of their ligands and functions. In the process of database search using the conserved sequence in the Ig domain that is shared between pIgR and Fcα/μR as reference, we noticed that the Ig V like domain of CD300LG has high homology with that of both receptors, and that CD300LG seems to be expressed specifically in certain tissues. Intriguingly, CD300LG is the most distant relative of the CD300L family members, and is well conserved in mouse and human. In this paper, we report the molecular characterization of CD300LG and its unique localization. In addition, its putative ligand and function will be discussed.

Materials and methods

Animals. C57BL/6J mice between 7 and 10 weeks old were obtained from CLEA Japan. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals in RIKEN.

cDNA clones and plasmid construction. An IMAGE consortium cDNA clone (clone ID No. 4913418) covering the coding sequence of mouse CD300LG-α was obtained from Invitrogen Corp., Carlsbad, CA. To obtain other isoforms of mouse CD300L, oligo(dT) primed cDNAs of the mouse heart were amplified by polymerase chain reaction (PCR) with a set of mouse-CD300LG-specific primers (5′-GCGGATCCACCATGAGGC CTCTGGT-3′ and 5′-CTGCAATTACACAGAGATGAAC-3′). The 0.7–1.0 kbp amplified fragments were subcloned into pBluescript-II KS(+) vector (Stratagene, Garden Grove, CA) and cDNA sequences were confirmed. These cDNA clones were identical to several RIKEN FANTOM cDNA clones and ESTs; mouse CD300LG-α: AK009375, -β: XM_126512, -γ: AK037204, -δ: AK052816 [14]. The cDNAs of two human CD300LG isoforms were obtained from Invitrogen Corp.; human CD300LG-γ and -δ: clone ID Nos. 5273562 and 4827737. Human CD300LG-α cDNAs were found in GenBank as TREM-4 α (AF427619) and β (AF427620). Mouse pIgR and Fcα/μR cDNAs were amplified from mouse intestine and spleen cDNAs, respectively, by PCR with a set of oligonucleotide primers synthesized on the basis of their published sequences (NM_011082, NM_144960) [15,16].

Mammalian expression vectors containing the above-obtained cDNAs were constructed by subcloning each cDNA fragment covering the entire coding sequence into the multicloning site of pcDNA3 (Invitrogen Corp., Carlsbad, CA) and pcDNA3-HAC, as described previously [17].

To produce mAbs against CD300LG, cDNA fragments corresponding to the extracellular domains of mouse CD300LG-α and human CD300LG-δ were amplified by PCR using the following primers (5′-GCG GATCCACCATGAGGCCTCTGGT-3′ and 5′-CCGGTCGACGGCCA TCATGCGGACCAT-3′ for mouse CD300LG-α; 5′-GGAGATCTACC ATGCGGCTTCTGGTCCTGC-3′ and 5′-CCGCTCGAGTATGCGGA CCATCGGGAT-3′ for human CD300LG-δ). The obtained cDNA fragments were inserted into the *Bam*HI/*Xho*I cloning sites of a modified pcDNA3 expression vector (pcDNA3-Fc) containing an *Xho*I/*Xba*I fragment coding the Fc segment of human IgG1.

Northern blot analyses. Multiple tissue RNA blots (TaKARA Bio Inc., Clontech, CA) of mouse and human were probed with ^{32}P-labeled cDNA fragment of mouse and human CD300LG, respectively, according to the instructions of the manufacturer. The probes used were: mouse CD300LG, a 573-bp fragment (nucleotides 44–616) coding for mouse CD300LG-α from the initial Met to 191 Ala; human CD300LG, an 832-bp fragment (nucleotides 206–1037) coding for human CD300LG-δ from 56 Ile to the terminal Ala. The human β-actin cDNA control probe was provided by the manufactures.

Antibodies. Polyclonal mouse CD300LG antisera were raised in two rabbits against a COOH-terminal 19-amino acid peptide conjugated with keyhole limpet hemocyanin, and one (#B3496) of them worked well. The polyclonal antiserum was purified with a protein A Sepharose column. Similarly, polyclonal human CD300LG antisera were also raised.

Anti-mouse CD300LG mAbs were generated by fusing the P3U1 myeloma cell line with popliteal lymph node cells from Wistar rats that had been immunized by injecting into their footpads the purified extracellular protein of mouse CD300LG-α that was fused with the Fc portion of human IgG1.

Goat polyclonal Abs against mouse CD31 (PECAM-1) and mouse Flt-4 (VEGFR3) were purchased from Santa Cruz Biotechnology, CA and R&D Systems, MN, respectively. Rat anti-HA mAb (3F10) was purchased from Roche Diagnostics. Human IgA1 and IgA2 were purchased from Athens Research and Technology, GA. Mouse IgA (TEPC15) and fluorescein (FITC)-labeled secondary Abs were from ICN Pharmaceuticals, CA. Mouse IgG and the other secondary Abs were from Jackson ImmunoResearch Laboratories, West Grove, PA.

Western blot analysis. pcDNA3-based expression vectors for each isoform of mouse CD300LG were transfected into HEK293T cells using FuGene6 transfection reagent (Roche Diagnostics, Indianapolis, IN). After 48 h, the cells were washed twice with ice-cold PBS and lysed with ice-cold cell lysis buffer (50 mM Tris–HCl, pH 7.5, 2.5 mM EDTA, 250 mM NaCl, 1% NP-40) containing Complete™ protease inhibitor mixture (Roche Diagnostics) by repeated vortexing. The homogenates were clarified by centrifugation at 10,000*g* for 10 min at 4 °C.

For preparation of lysates of mouse tissues, excised tissues were washed twice with ice-cold PBS and lysed with the ice-cold lysis buffer described above by 5×30 strokes on a Dounce homogenizer. The homogenates were clarified by centrifugation at 10,000*g* for 10 min at 4 °C. The lysates were boiled in SDS–PAGE sample buffer (10 mM Tris–HCl, pH 6.8, 1% SDS, 1% β-mercaptoethanol, 0.04% bromophenol blue, 20% glycerol), electrophoresed on 12.5% SDS–polyacrylamide gel, and electroblotted onto an Immobilon-P membrane (Millipore, Billerica, MA). The blot was incubated sequentially with rabbit anti-CD300LG polyclonal Ab and HRP-conjugated anti-rabbit IgG secondary Ab, and detected with a Super Signal West Pico (Pierce Chemical Co., Rockford, IL) by LAS-3000 mini (Fuji Photo Film, Japan).

Immunohistochemistry. For CD300LG immunostaining, 1% zinc sulfate/4% formalin-perfusional fixed sections of mouse tissues were deparaffinized, rehydrated, and treated with 0.3% H_2O_2 in PBS for 20 min at room temperature to block endogenous peroxidase activity [18]. Sections were incubated with 0.5% blocking buffer (Roche Diagnostics) in PBS for 30 min at room temperature and then incubated overnight at 4 °C with 1.25 μg/ml rabbit anti-mouse CD300LG polyclonal Ab, goat anti-mouse CD31 or Flt-4 polyclonal Ab. The binding of primary Ab was detected with biotinylated secondary Abs followed by streptavidin–horseradish peroxidase (ABC Elite; Vector Laboratories, Burlingame, CA) visualized with 3,3′-diaminobenzidine (DAKO Cytomation, Glostrup, Denmark), and counterstained with hematoxylin. For immunofluorescence staining, bound primary Abs were visualized with tyramid-FITC or -Cy3 (Perkin-Elmer, Boston, MA), followed by DAPI nuclear staining. The specimens were analyzed with a DM-IRE2 confocal laser scanning microscope and Leica confocal software (Leica Microsystems, Mannheim, Germany).

Immunoelectron microscopy. For immunoelectron microscopy, the preembedding silver enhancement immunogold method was performed as previously described [19]. Mouse hearts were fixed by transcardially perfusing 4% paraformaldehyde in 0.1 M Na-phosphate buffer, pH 7.4 (PB), for 10 min. Cryosections of the hearts (6 μm in thickness) were prepared and incubated for 30 min in PB containing 0.005% saponin, 10%

bovine serum albumin, 10% normal goat serum, and 0.1% cold water fish skin gelatin for blocking. The sections were then treated with rabbit polyclonal antiserum against CD300LG in the blocking solution, overnight. Then, the sections were washed six times with PB containing 0.005% saponin for 10 min each and incubated with goat anti-rabbit IgG conjugated to colloidal gold (1.4 nm diameter) in the blocking solution for 2 h. The samples were washed six times with PB for 10 min each and fixed with 1% glutaraldehyde in PB for 10 min. After washing, the gold labeling was intensified by using a silver enhancement kit (HQ Silver, Nanoprobes Inc., NY) for 6 min at 20 °C in the dark. After washing with distilled water, the cells were postfixed in 0.5% OsO_4 for 90 min at 4 °C, washed with distilled water, dehydrated once with a series of graded ethanol solutions (30%, 50%, 70%, 90% ethanol for 10 min each) and twice with 100% ethanol, and then embedded in epoxy resin. Ultrathin sections were doubly stained with uranyl acetate and lead citrate and observed under an H7600 electron microscope (Hitachi, Tokyo, Japan).

Transcytosis assay. Madin-Darby canine kidney (MDCK) cells were grown in Dulbecco's modified essential medium (Sigma–Aldrich, St. Louis, MO) containing 10% fetal bovine serum at 37 °C, 5% CO_2. The pcDNA3-based expression vectors for each isoform of mouse CD300LG were transfected into MDCK cells using FuGene6 transfection reagent. Cells transiently expressing mouse CD300LG were plated on polycarbonate membrane filters at a density of 1×10^5 cells/12-mm filter (Transwell units, 0.4-μm pore size, Corning Coster Corp., Corning, NY). After incubation for 4 days, the cells were pre-incubated for 20 min at 4 °C. Subsequently, anti-CD300LG mAbs were added to either the apical or the basolateral side. After incubation for 1 h at 4 °C, the temperature was increased to 37 °C for additional incubation for 1 h. The cells were washed twice with PBS and fixed with 4% paraformaldehyde/PBS for 30 min at room temperature, permeabilized with 0.1% Triton X-100/PBS, washed twice with PBS and stained with Cy-3-anti-rat IgG and Cy5-labeled phalloidin. The stained cells were observed with a confocal laser scanning microscope.

Ig uptake assay. HeLa cells were transfected with the expression vectors for each isoform of CD300LG tagged with the C-terminal HA-epitope, using FuGene6 transfection reagent. The transfected cells were cultured for 24 h and then incubated for 1 h at 37 °C with mouse IgA, human IgA1, human IgA2, mouse IgM, or mouse IgG. The cells were washed twice with PBS and fixed with 4% paraformaldehyde/PBS for 30 min at room temperature, permeabilized with 0.1% Triton X-100/PBS, and incubated for 1 h at room temperature with anti-HA (3F10) Ab. The cells were washed twice with PBS and stained with Cy3-labeled anti-rat IgG and the corresponding FITC-labeled anti-Ig isotype. The stained cells were observed with the confocal laser scanning microscope as described above.

Results

Cloning and molecular characterization of CD300LG

To identify the novel Fc receptors for IgA, we made use of large databases of ESTs with Ig domains to identify those which showed homology with a region of the Ig domain conserved significantly between pIgR and Fcα/μR. As a result, we found a mouse Ig receptor like gene that encodes a putative type-I membrane protein containing one Ig domain having approximately 35% identity to those of pIgR and Fcα/μR, respectively. The gene was named CD300 antigen like family member G (CD300LG) or CMRF35 antigen like molecule 9 (CLM-9) [9], but no further descriptions have been published. To characterize this gene, we firstly cloned the full-length cDNAs of mouse CD300LG (Fig. 1A). The CD300LG gene is located on mouse chromosome 11D, and is composed of eight exons. We identified four isoforms and named them CD300LG-δ, -γ, -β, and -α in the order of the length of the deduced amino acid sequences, 415, 371, 331, and 287 aa, respectively. Compared with the longest δ form cDNA, the γ-isoform lacks exon 3, the β-isoform lacks about two-thirds of exon 4, and the α-isoform lacks both of them (Fig. 1B). Further database search revealed the human counterpart of mouse CD300LG that is encoded on human chromosome 17q21. The human CD300LG comprised at least four isoforms, and their genomic structures were similar to that of the mouse ortholog. Of note is that all of the human CD300LG cDNAs so far identified have a shorter cytoplasmic tail that lacks a region corresponding to exon 7 of mouse CD300LG. Moreover, there are two human counterparts for the mouse α-isoform, which are diverse in their C-terminal regions and are referred to as TREM-4 α and -β on the database. We have no direct evidence so far of the existence of the human ortholog of mouse β-isoform, since we were not able to clone the human β-isoform and there has been no database entry corresponding to the human β-isoform.

Northern blot analyses were next performed to examine the tissue distribution of CD300LG (Fig. 2). Mouse CD300LG mRNAs were expressed highly in the heart, moderately in a variety of tissues, and slightly in the spleen and the thymus, and not at all in the brain. As shown in Fig. 2B, three mRNA bands (1.7, 3.0, and 3.4 kb) were detected in all the tissues expressing CD300LG. A similar expression pattern was observed with human tissues (Fig. 2A); CD300LG mRNAs were expressed intensely in the heart, the skeletal muscle and the placenta, and faintly in some tissues, but were not expressed in the brain.

To characterize CD300LG at the protein level, antisera were raised in rabbits against a synthetic peptide corresponding to the C-terminal 19 amino acids of mouse CD300LG proteins. To examine the specificity of the antisera, Western blot analysis was performed on the lysates of HEK293T cells transfected with the expression vector for each isoform of mouse CD300LG (Fig. 3, right). All four isoforms were detected as several bands: bands whose sizes correspond to the calculated molecular weights (Fig. 3, asterisks), and large ones likely reflecting glycosylation. The antisera also detected endogenous CD300LG expressed in mouse tissues (Fig. 3, left). All isoforms could be found in the heart, where the shortest α-isoform was expressed preferentially. In the kidney and the liver, by contrast, the β-isoform was predominant, indicating that tissue-specific regulation could exist for the expression of CD300LG isoforms.

CD300LG is exclusively expressed on capillary endothelium

To identify the cellular localization of CD300LG, we performed immunohistochemical analysis of mouse tissue using anti-mouse CD300LG antisera. Interestingly, CD300LG was localized on capillaries in the mouse heart (Figs. 4A, C, and E). To determine the localization of CD300LG more precisely, we compared the expression

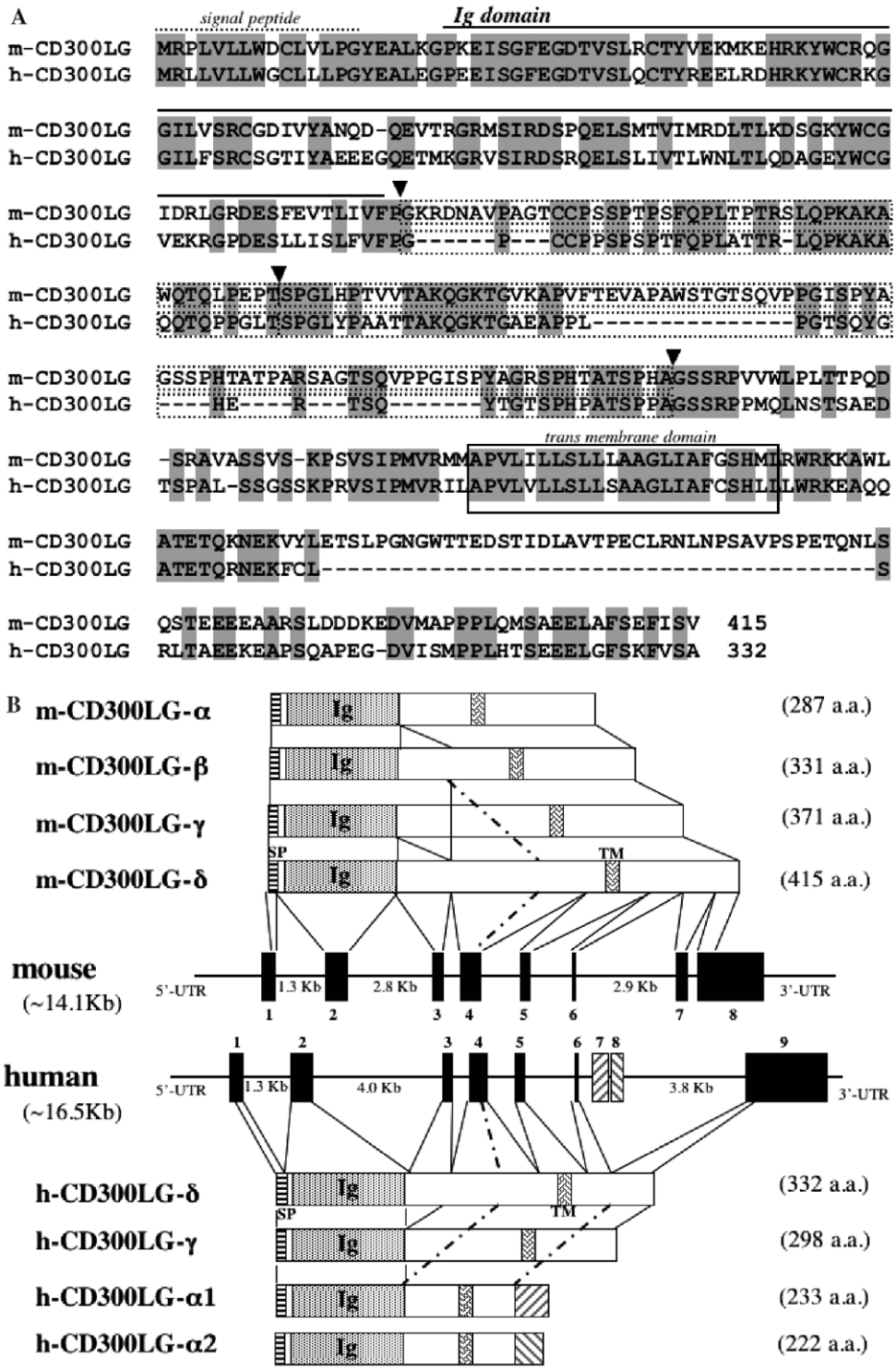

Fig. 1. Comparison of deduced amino acid sequences of mouse and human CD300LG-δ isoforms. (A) The overall amino acid sequences of mouse and human CD300LGs show 51% homology. Conserved residues between the two species are shaded, putative transmembrane domains are enclosed in a box, and deleted regions in spliced variants are indicated by broken line boxes. Arrowheads indicate sites of alternative splicing. The positions of the signal peptide and the Ig domain are also indicated. The cytoplasmic tail of human CD300LG is shorter than that of mouse. Polyclonal anti-mouse CD300LG antisera were raised in rabbits against a COOH-terminal 19-amino acid peptide. Rat anti-mouse CD300LG mAbs were generated against a chimeric protein corresponding to the extracellular region of mouse CD300LG-α fused with the Fc portion of human IgG. (B) Schematic representation of the splicing variants of CD300LG. At least four CD300LG isoforms exist in both mouse and human. Mouse CD300LG-δ is composed of eight exons. Mouse γ-isoform and β-isoform lack exon 3 and about two-thirds of exon 4, respectively. Mouse α-isoform lacks both of them. Human CD300LGs have a shorter cytoplasmic tail that lacks a region corresponding to exon 7 of mouse CD300LG. There are two human counterparts for the mouse α-isoform in which the COOH-termini diverge from each other by alternative splicing. There is no evidence for the existence of the human ortholog of mouse β-isoform (SP, signal peptides; TM, transmembrane domain; Ig, immunoglobulin domain).

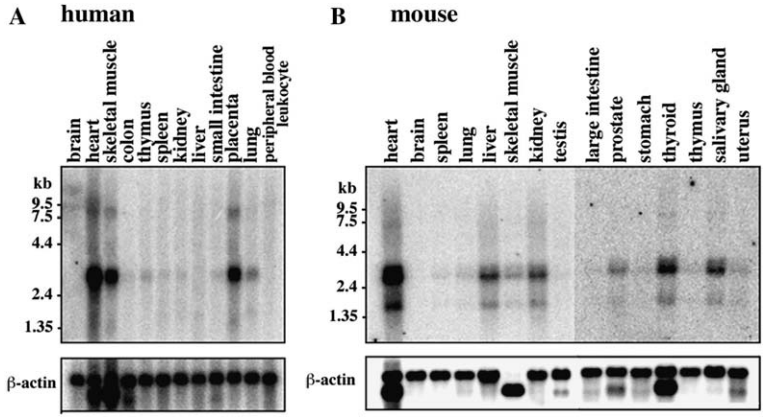

Fig. 2. Northern blot analyses of human and mouse CD300LGs. Human (A) and mouse (B) multiple RNA blots were analyzed with corresponding CD300LG cDNA fragments, respectively. Each fragment covered all cognate isoforms (upper panels). The blots were then stripped and reprobed with the β-actin probe (lower panels).

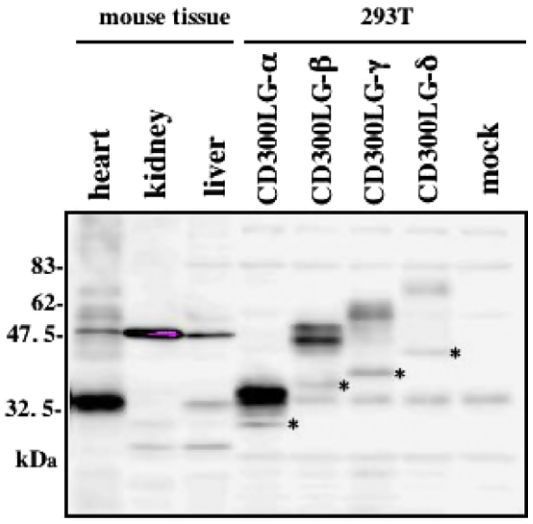

Fig. 3. Biochemical analysis of endogenous and exogenous mouse CD300LG proteins. Total lysates from mouse tissues, untransfected HEK293T cells (mock) and cells transiently expressing each of mouse CD300LG isoforms were prepared with lysis buffer containing 1% Triton X-100 as described in Materials and methods, and subjected to Western blotting. The blot was probed with polyclonal anti-mouse CD300LG antisera. Asterisks indicate bands whose sizes correspond to the calculated molecular weights.

patterns of CD300LG and CD31 in serial sections. CD31 is a general marker expressed on endothelial cells of all blood vessels [20,21]. In contrast, CD300LG was localized only on capillaries and not on large blood vessels (Figs. 4C and D). The staining pattern of CD300LG was also different from that of Flt-4, which is expressed on lymph vessels (Figs. 4E and F) [22]. These results confirm the capillary-endothelium-specific expression of CD300LG in the mouse heart. The capillary-endothelium-specific localization of CD300LG was also observed in other mouse tissues including the liver and the tongue (data not shown).

We further performed immunoelectron microscopy to examine the subcellular localization of CD300LG on the capillary endothelium in the mouse heart. CD300LG was localized on both apical and basolateral plasma membranes (Fig. 5). Some CD300LG signals were also found on intracellular vesicular structures (Fig. 5, arrows in inset). These results imply that CD300LG might be transported across the cytoplasm in the capillary endothelium.

CD300LG is transcytosed constitutively in polarized MDCK cells

To test the hypothesis that CD300LG is transported across the cytoplasm, we utilized MDCK cells transfected with mouse CD300LG and mAbs that were raised against the ectodomain of mouse CD300LG. First, we tried to use such endothelial cell lines as human umbilical endothelial cells (HUVEC), human microvascular endothelial cells (HMEC-1) and mouse lymphoid endothelial cells (SVEC4-10) for the transcytosis assay [23,24]. However, these endothelial cells were not appropriate because they were too thin and flat to enable clear distinction of the apical and basolateral localizations of CD300LG at the available resolution of the immunofluorescence microscope (HT and HO, unpublished observation). Alternatively, we chose MDCK cells for this assay, since valuable information about transcytosis in polarized cells has emerged based largely on studies using MDCK cells. When MDCK cells transfected with mouse CD300LG-δ and cultured on polycarbonate membrane filter were incubated with anti-CD300LG mAb added from the apical side for 1 h at 4 °C, the Abs stayed on the apical plasma membrane (Fig. 6A–A″). By contrast, after 1 h incubation at 37 °C, the Abs were detected intracellularly as well as on the basolateral plasma membrane (Fig. 6B–B″). Similar results were obtained when the Abs were added from the basal side (Figs. 6C and D). We also observed that the human ortholog showed similar characteristics to mouse CD300LG (not shown). These results indicate that CD300LG is transcytosed across polarized MDCK cells in a bidirectional manner, implying that a similar transcytosis may also take place in endothelial cells.

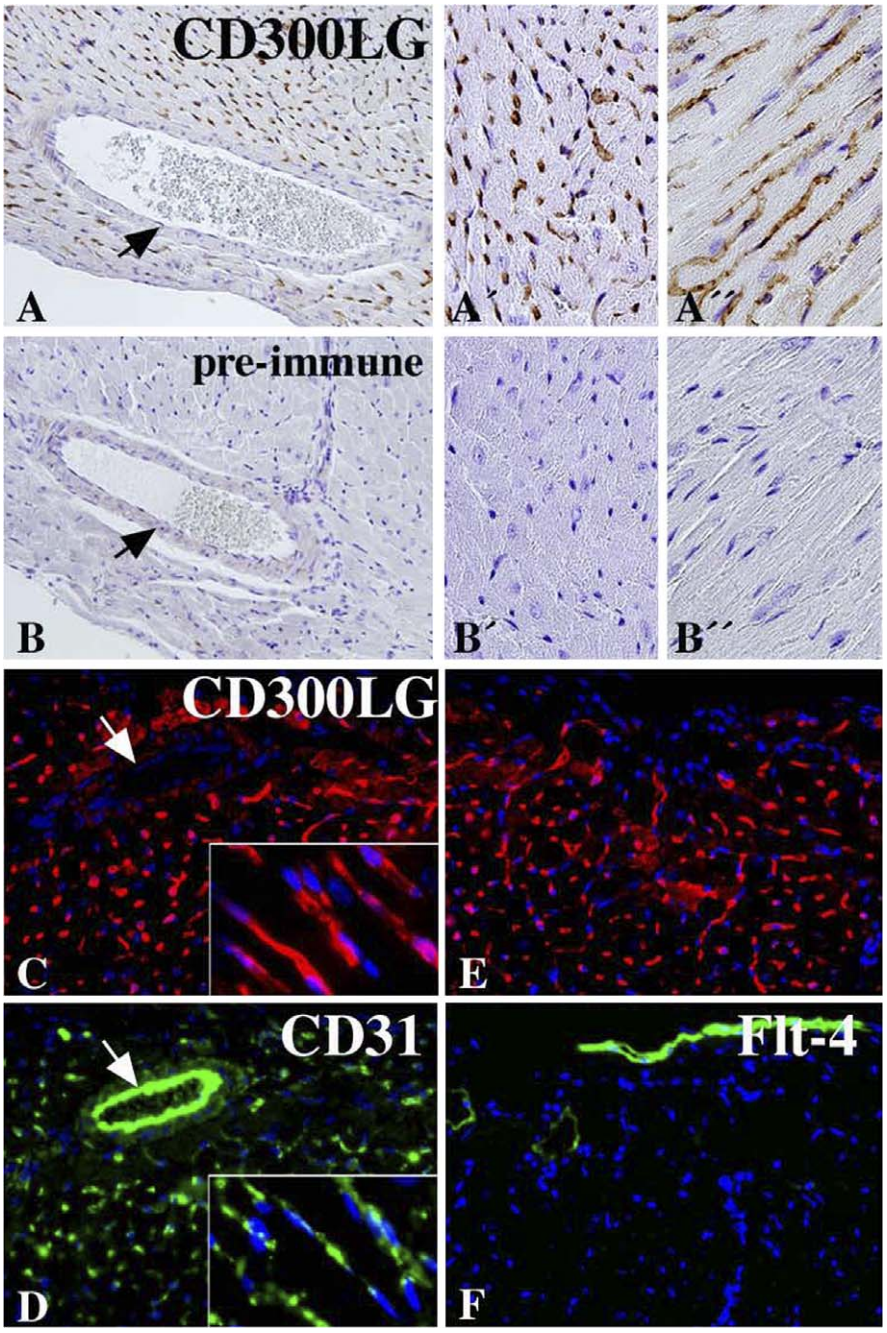

Fig. 4. Localization of CD300LG in mouse heart. Paraffin sections of mouse heart were reacted with polyclonal rabbit anti-mouse CD300LG antiserum (A–A″, C and E), pre-immune serum as control (B–B″), goat anti-mouse CD31 (D) or goat anti-mouse Flt-4 (F) Abs. Binding of primary Ab was detected with biotinylated secondary Abs followed by streptavidin–horseradish peroxidase and 3,3′-diaminobenzidine (A–A″ and B–B″), tyramid-Cy3 (C and E) or tyramid-FITC (D and F) as described in Materials and methods. Arrows indicate blood vessels (larger than capillary). (A,B), (C,D) or (E,F) show staining on serial sections, respectively. Sections were counterstained with DAPI (blue) (Magnification: 200×, A, B, C–F; 400×, A′, A″, B′, B″, insets in C and D).

CD300LG binds and internalizes human IgA2 and mouse IgM on HeLa cells

Since CD300LG was initially identified as a molecule possessing the Ig domain that shares sequence homology with those of pIgR[10] and Fcα/μR, we next examined whether the CD300LG proteins could interact with Igs. As the MDCK cell subpopulation possesses intrinsic IgA binding capacity (HT and HO, unpublished observation), we could not use it to test Ig uptake. To this end, HeLa cells were transfected with each isoform of mouse CD300LG tagged with the HA-epitope, and incubated with mouse IgA, human IgA1, human IgA2, mouse IgM, or mouse IgG, to test their uptake. After 1 h incubation at 37 °C, the cells were fixed and processed for indirect immunofluorescence analysis. Mouse CD300LG-α was detected with anti-HA Abs, as shown in Fig. 7. Cells expressing mouse CD300LG-α were able to take up human

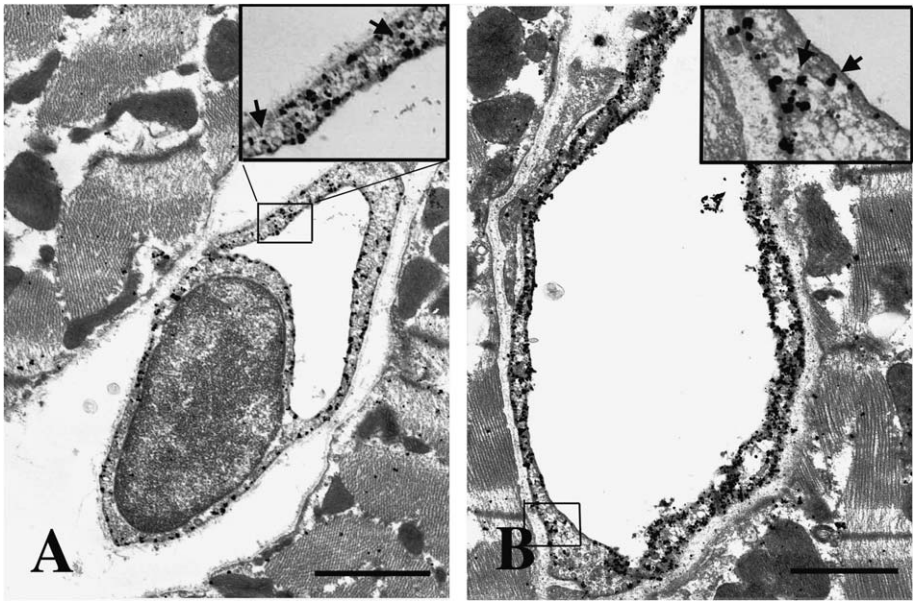

Fig. 5. Ultrastructural analysis of CD300LG localization by immunoelectron microscopy. Mouse heart cryosections were processed for immunogold labeling with polyclonal rabbit anti-CD300LG antiserum as described in Materials and methods. Scale bars represent 1 μm. Arrows in high magnification insets indicate intracellular vesicular structures.

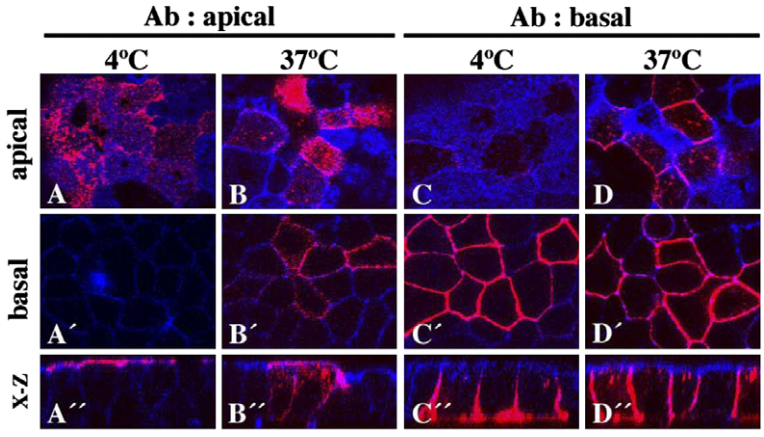

Fig. 6. Uptake of anti-CD300LG mAb by MDCK cells expressing mouse CD300LG. MDCK cells were transfected with mouse CD300LG-δ, cultured on polycarbonate membrane filters, and incubated with anti-CD300LG mAb from the apical (A–A″, B–B″) or basal (C–C″, D–D″) side for 1 h at 4 °C. In B–B″ and D–D″, cells were further incubated for 1 h at 37 °C. The cells were then fixed, permeabilized and stained with Cy-3-anti-rat IgG and Cy5-labeled phalloidin as described in Materials and methods. Shown are the horizontal (X–Y) sections at the apical (A–D) or basolateral (A′–D′) plasma membrane and the vertical (X–Z) sections (A″–D″).

IgA2 and mouse IgM efficiently (Figs. 7C and D). However, CD300LG showed slight co-localization with internalized Igs. Mouse IgA and human IgA1 were also internalized but less efficiently, whereas mouse IgG did not bind to CD300LG. Similar results were also observed with the other isoforms and the human ortholog (data not shown). Together, these observations imply that IgA and IgM may be candidates for CD300LG ligands.

Discussion

In the present study, we demonstrated that CD300LG shows restricted expression on capillary endothelium in mouse, in contrast to the fact that many genes of this family are expressed on a variety of hematopoietic cell types [9–11,25–29]. And human CD300LG transcripts were expressed strongly in the human heart and placenta where capillaries are highly developed. In mouse and human, the CD300L family is located on chromosomes 11 and 17, respectively. Whereas CD300L(A–F) genes exist as a cluster in close proximity, the CD300LG locus is distant from them, albeit being found on the same chromosome, in both species. The CD300L family, except for CD300LG, is generally considered to be signaling receptors in hematopoietic cells, playing regulatory roles in immune response [9,26]. Considering the differences in tissue distribution

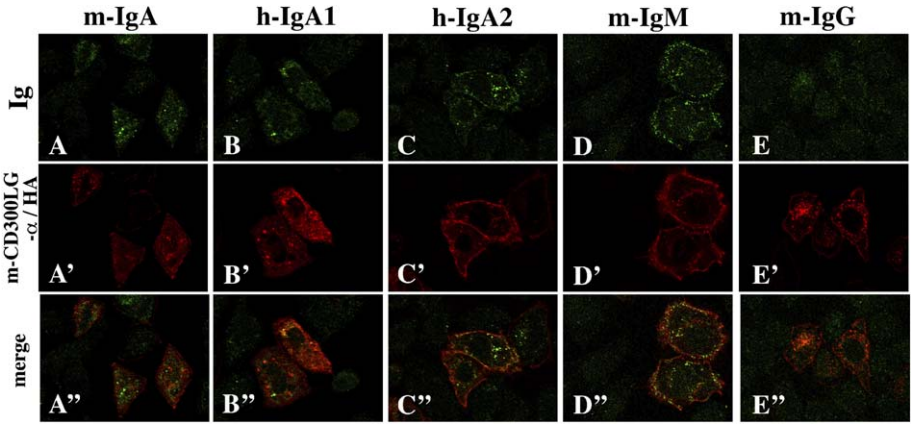

Fig. 7. Uptake of Igs by HeLa cells exogenously expressing mouse CD300LG-α. HeLa cells were transfected with expression vectors of HA-tagged mouse CD300LG and incubated with indicated Igs for 1 h at 37 °C. Bound/and or internalized Igs were detected with FITC-conjugated secondary Ab against each Ig class as described in Materials and methods (A–E). Exogenously expressed CD300LG was stained with anti-HA (3F10) Ab in combination with Cy3-conjugated secondary Ab (A′–E′). A″–E″ are merged images of A–E and A′–E′.

and genetic structure, CD300LG may have a function distinct from other members of the CD300L family.

The bidirectional transcytosis of CD300LG observed in MDCK cells suggests that it might also be transcytosed across polarized endothelial cells in a bidirectional manner. Ultrastructural analysis using immunoelectron microscopy revealed the localization of CD300LG on both the apical and basolateral plasma membranes of the capillary endothelium, with some also residing on the intracellular vesicular structures. These observations suggest that CD300LG is transcytosed in the capillary endothelium. We also observed that CD300LG expressed in HeLa cells was able to bind and internalize mouse and human IgA as well as mouse IgM, although the affinity seemed very low except that for human IgA2. Since IgG failed to bind or be endocytosed in a CD300LG-dependent manner, we suppose that IgA and IgM may be the candidate ligands for CD300LG. These results, however, do not necessarily negate the possibility that CD300LG could have yet other ligand(s), especially considering its relatively weak affinity other than that for human IgA2. In this sense, it may be intriguing to assume that CD300LG isoforms could recognize distinct ligands to be transported across the capillary endothelium with their extracellular ''stalk''-like region connecting the Ig domain and the transmembrane domain, where the isoforms are structurally different because of alternative splicing. As regards Ig receptors on endothelium, the neonatal Fc receptor (FcRn) is well known [30]: Its function as a transcytotic receptor that ferries maternal IgG across cells into the fetal and/or neonatal bloodstream can be extended to IgG homeostasis. CD300LG might have an analogous function to FcRn, for IgA and IgM.

During the preparation of this manuscript, Umemoto et al. have published a paper describing a novel high endothelial venules (HEV) sialomucin. They named the molecule nepmucin (mucin not expressed in Peyer's patch) because it is expressed in lymph node LN HEVs but not detectable in Payer's patch HEVs at the protein level. They showed that nepmucin mediates L-selectin-dependent lymphocyte rolling and promotes lymphocyte adhesion under physiological conditions [31]. Surprisingly, nepmucin is identical to CD300LG. Interestingly, it is implicated that nepmucin serves as a functional ligand for L-selectin and mediates lymphocyte rolling when its mucin-like domain is appropriately glycosylated by a specific set of carbohydrate-modifying enzymes in LN HEVs. Taken together with the fact that nepmucin/CD300LG is strongly expressed in the capillary endothelium of various tissues as well, the molecule likely has multiple functions and may play a different role in capillaries rather than lymphocyte rolling and adhesion. Further studies, including the establishment and analysis of CD300LG-knockout mice currently underway in our laboratory, will be required to shed light on the entire physiological significance of the protein.

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