Molecular Cloning of a Novel Human Collectin from Liver (CL-L1)*

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Collectins are a C-lectin family with collagen-like sequences and carbohydrate recognition domains. These proteins can bind to carbohydrate antigens of microorganisms and inhibit their infection by direct neutralization and agglutination, the activation of complement through the lectin pathway, and opsonization by collectin receptors. Here we report the cloning of a cDNA encoding human collectin from liver (CL-L1 (collectin liver 1)) that has typical collectin structural characteristics, consisting of an N-terminal cysteine-rich domain, a collagen-like domain, a neck domain, and a carbohydrate recognition domain. The cDNA has an insert of 831 base pairs coding for a protein of 277 amino acid residues. The deduced amino acid sequence shows that this collectin has a unique repeat of four lysine residues in its C-terminal area. Northern blot, Western blot, and reverse transcription-polymerase chain reaction analyses showed that CL-L1 is present mainly in liver as a cytosolic protein and at low levels in placenta. More sensitive analyses by reverse transcription-polymerase chain reactions showed that most tissues (except skeletal muscle) have CL-L1 mRNA. Zoo-blot analysis indicated that CL-L1 is limited to mammals and birds. A chromosomal localization study indicated that the CL-L1 gene localizes to chromosome 8q23-q24.1, different from chromosome 10 of other human collectin genes. Expression studies of fusion proteins lacking the collagen and N-terminal domains produced in Escherichia coli affirmed that CL-L1 binds mannose weakly. CL-L1 and recombinant CL-L1 fusion proteins do not bind to mammalian columns. Analysis of the phylogenetic tree of CL-L1 and other collectins indicated that CL-L1 belongs to a fourth subfamily of collectins following the mann-binding protein, surfactant protein A, and surfactant protein D subfamilies including bovine conglutinin and collectin-43 (CL-43). These findings indicate that CL-L1 may be involved in different biological functions.

EXPERIMENTAL PROCEDURES

Buffers and Media—Escherichia coli lysis buffer for the pMAL-c2 system contained 10 mM phosphate, pH 7.2, 30 mM NaCl, 0.25% (w/v) Tween 20, 10 mM 2-mercaptoethanol, 10 mM EDTA, and 10 mM EGTA. Column buffer contained 10 mM phosphate, pH 7.2, 500 mM NaCl, 1 mM NaN4, 10 mM 2-mercaptoethanol, and 1 mM EDTA, and column buffer/T contained column buffer and 0.25% (w/v) Tween 20. E. coli lysis buffer A for the His tag system contained 6 mM guanidine hydrochloride, 0.1 M sodium phosphate, and 10 mM Tris, pH 8.0. Column buffers B-E consisted of 8 M urea, 0.1 M sodium phosphate, and 10 mM Tris, with pH values of 8.0, 6.3, 5.9, and 4.5, respectively. LB medium contained 1% (w/v) Bacto-Tryptone, 0.5% (w/v) Bacto-yeast extract, and 1% (w/v) NaCl. Induction base medium with glucose contained 0.4% casamino acids, 0.6% NaHPO4, 0.3% KH2PO4, 0.05% NaCl, 0.1% NH4Cl, 0.5% glucose, and 1 mM MgCl2. Tris-buffered saline (TBS) consisted of 20 mM Tris-HCl and 140 mM NaCl, pH 7.4, and TBS/C was TBS containing 5 mM CaCl2. Coating buffer contained 15 mM Na2CO3, 35 mM NaHCO3, and 0.05% (w/v) NaN3, pH 9.6.

Generation of a Probe for Screening by Polymerase Chain Reaction—Screening an expressed sequence tag (EST) data base for potential new collectin genes revealed a novel gene in EST clone R29493. The partial

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The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBankTM/EMI Data Bank with accession number AB002631.

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Clone (F1-1006D) from a fetal liver cDNA was kindly provided by Dr. Hee-Sup Shin (Pohang Institute of Science and Technology) and used to screen a human liver cDNA library for full-length cDNAs by plaque hybridization. To generate a digoxigenin-labeled cDNA probe, we used the polymerase chain reaction (PCR). Primers amplifying the cDNA product were synthesized based on the nucleotide sequences of the insert in clone F1-1006D. The primers synthesized were 5'-GGCGCAACACAGCTGACG-3' for the reverse primer and 5'-TTATTTTTCCTCTGTTG-3' for the forward primer. PCR was carried out using a PCR DIG labeling kit (Roche Molecular Biochemicals). The reaction mixture (50 μl) consisted of 10 μM Tris-HCl, pH 8.3, 50 μM KCl, 20 μM each of dATP, dCTP, and dGTP, and 20 μM each of dGTP and digoxigenin-11-dUTP; 1.25 units of Taq DNA polymerase; and 1 μl of human liver genomic DNA. PCR was performed for 30 cycles in a TakaRa PCR Model 480 thermal cycler (Takara Shuzo Co., Ltd.), with each cycle consisting of denaturation for 45 s at 95 °C, annealing for 1 min at 60 °C, and extension for 2 min at 72 °C. The PCR product was electrophoresed on a 1% (w/v) agarose gel (Wako Pure Chemical Industries) and then directly subcloned into the pCRT2.1 vector of a TA cloning kit (Takara Shuzo Co., Ltd.). The plasmids were sequenced using an Autoread DNA sequencing kit and 0.1% SDS at room temperature and then for 15 min in 0.1× SSC and 0.1% SDS at 68 °C. The hybridized probe was detected as described above.

Reverse transcription was carried out using total RNAs (1 μg) from human liver, kidney, lung, tongue, brain, heart, intestine, spleen, stomach, testis, mammary gland, prostate, skeletal muscle, testis, uterus, placenta, adrenal gland, pancreas, salivary gland, and thyroid. Oligo(dT)-adaptor primers (RNA LA PCR kit (avian myeloblastosis virus), Version 1.1, Takara Shuzo Co., Ltd.) were used for the reverse transcription reaction. The reverse transcription products were amplified by 28 or 35 cycles of PCR using degenerate first primers (0.2 μg), TaKaRa LA Tag polymerase (1.25 units), and reverse transcription reaction products in a TaKaRa PCR Model MP thermal cycler. Nested PCRs were performed using the PCR products as a probe after 35 cycles of RT-PCR. The primer set for the first PCR was 5'-TAGCAATACGTTAGGATGAG-3' for the reverse primer and 5'-CCAAGCAAAGTATGCGCA-3' for the forward primer. The inner primer set for nested PCR was 5'-TTATTTTTCCTCTGTTG-3' for the reverse primer and 5'-ATGATGGCTGCTCTTTC-3' for the forward primer. These primers were located in the neck and CRD regions, which spanned an intron in genomic DNA. Amplicons were separated on 1% agarose gels.

Chromosomal Localization of the CL-L1 Gene—The ~10-kilobase pair human CL-L1 genomic DNA fragment from the neck domain to the CRD showed a hybridization signal to a nick-translated labeled probe similar to a genomic probe specific to the E. coli chromosome (Roche Molecular Biochemicals), was used as a probe. The gene was localized by fluorescence in situ hybridization and PCR analysis using DNAs from human monochromosomal fusion cells kindly provided by Dr. Hashimoto (National Institute of Infectious Diseases). Map position was determined by inspection of fluorescent signals on 4,6-diamidino-2-phenylindole-stained chromosomes. 25 metaphase preparations were analyzed.

Zoo-blot analysis—To generate a digoxigenin labeled cDNA probe for the zoo-blot analysis, a human equivalent of the 5'-end nucleotide sequence of the insert in clone F1-1006D (which corresponded to the CRD) was made using a PCR DIG labeling kit (Roche Molecular Biochemicals). The probe was a whole cDNA insert subcloned into pSPT18. The filters were washed twice for 5 min in 2× SSC and 0.1% SDS at room temperature and then for 15 min in 0.1× SSC and 0.1% SDS at 68 °C. The hybridized probe was detected as described above.

Zoo-blot analysis was performed with the modified method described above using completely EcoRI-digested genomic DNAs (5 μg) from human, rhesus monkey, cow, dog, rabbit, rat, mouse, chicken, and yeast (Saccharomyces cerevisiae) as targets. Gel blots were prehybridized in ExpressHyb hybridization solution (CLONTECH) at 68 °C for 30 min and then hybridized for 1 h at 68 °C with a 10 ng/ml concentration of the digoxigenin-labeled probe corresponding to a fragment of the CRD in the same buffer as used for prehybridization. The filters were washed for 5 min in 2× SSC and 0.1% SDS at room temperature and then for 15 min in 0.2× SSC and 0.1% SDS at 68 °C. The hybridized probe was detected by a chemiluminescent technique (Roche Molecular Biochemicals) as described by the manufacturer.
of lysis buffer A and lysed by sonication (15 s, 70% output, 10 times). After centrifugation at 9000 x g for 30 min, the supernatant was incubated with nickel-nitricatric acid-agarose (QIAGEN Inc.) for 15 min, and the gel was loaded onto a column. The column was washed with column buffers B and C. The histidine-tagged recombinant protein was eluted with 0.1 M imidazole buffer (pH 7.0). The eluate was against 1000 volume of TBS, followed by two changes of 1000 volumes of TBS/C. This CL-L1 fusion protein (CL-L1-CDRhis) was used to produce antisera in New Zealand White rabbits. Purification and identification of the recombinant CL-L1 CRDs were confirmed by SDS-PAGE and Western blotting using the rabbit anti-CL-L1 CRD serum.

**RESULTS**

Cloning of Recombinant CL-L1 CRDs Produced in E. coli—The binding of recombinant CL-L1-CDRhis to mannan (mannose-biotin probe) was measured by ELISA with avidin-biotinylated peroxidase (Vector Labs, Inc.). Microtiter plates were coated with 3,3'-tetramethylbenzidine substrate solution (3,3'-tetramethylbenzidine microwell peroxidase substrate, Kirkegaard and Perry Laboratories) and incubated with each step. Finally, 100 μl of coating buffer (15 mM Na2CO3, 35 mM NaHCO3, and 0.05% (v/v) NaCN, pH 9.6). The plates were washed three times with 20 mM Tris-HCl, pH 7.4, 140 mM NaCl, 0.05% NaN3, and 0.05% (v/v) Tween 20 after each step. After washing, the plates were blocked with Block Ace (Dainippon Seiyaku, Tokyo) for 1 h at room temperature. After blocking, the samples were supplemented with an α-α-mannose BP-probe (Seikagaku Kogyo, Tokyo) at a concentration of 0.01, 0.1, and 1 μg/ml without mannann (10 mM) or EDTA (10 mM). The samples were incubated with or without EDTA (10 mM) or EDTA (10 mM) alone or with EDTA (10 mM) or mannan (10 mM). The staining procedure was described above.

Another characterization was performed using a sugar-blot method (20). Recombinant CL-L1 CRDs, manlose-binding protein (1 μg of each) were dissolved in SDS sample buffer, separated by SDS-PAGE, and transferred to BioBlot-NC membranes (Coster Co.) by standard procedures. After treating the membranes with TBS/TC (20 mM Tris-HCl, 140 mM NaCl, 0.1% Triton X-100, and 5 mM CaCl2) with or without EDTA (10 mM), they were incubated with the α-α-mannose BP-probe alone or together with EDTA (10 mM) or mannan (100 μg/ml) at 4 °C for 1 h at each step. Finally, 100 μl of 3:3:5,5′-tetramethylbenzidine substrate solution (3:3:5:5′-tetramethylbenzidine microwell peroxidase substrate, Kirkegaard and Perry Laboratories) was added sequentially and incubated for 1 h at 4 °C after each step. The inhibition studies were done with EDTA (10 mM) and mannan (10 mg/ml). The lectins, α-α-mannose, α-α-mannose, α-N-acetylgalactosamine, and α-N-acetylgalactosamine BP-probes (Seikagaku Kogyo). The inhibition studies were done with EDTA (10 mM) and mannan (10 mg/ml).

**Conclusions**

The fluorescence images were observed with a Nikon Optiphot-2 microscope (Nikon Corporation). The micrographs were processed with Filing Imaging Software US6341 (Hamamatsu Photonics K.K.).

**Sequence Analysis and Construction of a Phylogenetic Tree**—The comparison of the amino acid sequence of the CRD of CL-L1 with the GenBank™ sequences below was done using the DNA Sequencer Analysis Program (Hitachi). A phylogenetic tree was constructed by the neighbor-joining method (22) using the amino acid sequences of the CRD of CL-L1. Human MBP (23), mouse MBP-A (24), mouse MBP-B (24), bovine MBP (17), rabbit MBP (25), rat MBP-C (3), rat MBP-A (3), mouse MBP-A (26), mouse MBP-C (26), bovine conglutinin (27), bovine SP-D (28), bovine CL-43 (29), rat SP-D (30), and human SP-A (4). The phylogenetic relationships were analyzed using the computer program PHYLIP Version 3.67rc package (25).

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**RESULTS**

Identification of a New Human Liver Collectin (CL-L1)—We screened DNA databases to identify novel members of the collectin family. This resulted in the identification of a cDNA fragment from human EST data bases that showed carboxy-terminal sequence homology to the collectins. The EST clone F1-1066D from a fetal liver cDNA library was used to screen the human liver cDNA library, and two positive clones (HL11-3M and HL11-9) were isolated. Furthermore, cap site hunting was performed to determine the complete 5'-terminal sequence including the transcriptional start site of CL-L1 mRNA using Cap Site cDNA™ (18). Restriction map analysis and sequencing of the clones revealed that they contained an open reading frame encoding a sequence of 277 amino acid residues. The cDNA contained a 75-nucleotide 5'-nontranslated sequence, followed by 831 nucleotides corresponding to a whole protein.
and a 759-nucleotide 3'-nontranslated sequence with an AATAA incomplete polyadenylation signal (Fig. 1). The deduced amino acid sequence from the cDNA revealed a collectin structure consisting of an N-terminal region with cysteine residues, a collagen-like region, a neck region, and a CRD. A hydrophobic signal peptide sequence was not evident at the N-terminal region, and the amino-terminal residue of the mature protein was unknown.

Northern Blot and RT-PCR Analyses—To examine the distribution of CL-L1 mRNA, Northern blot analyses were performed with mRNAs from various human tissues. The Northern blot analysis, poly(A)* RNAs (2 µg) from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas were subjected to 1% agarose gel electrophoresis. Calculated sizes of detected RNAs are indicated by arrowheads. B, RT-PCR analyses using total RNAs (1 µg) from brain (lane 1), heart (lane 2), kidney (lane 3), liver (lane 4), lung (lane 5), trachea (lane 6), bone marrow (lane 7), colon (lane 8), small intestine (lane 9), spleen (lane 10), stomach (lane 11), thymus (lane 12), mammary gland (lane 13), prostate (lane 14), skeletal muscle (lane 15), testis (lane 16), uterus (lane 17), placenta (lane 18), adrenal gland (lane 19), pancreas (lane 20), salivary gland (lane 21), and thyroid (lane 22) were carried out (28 or 35 cycles of RT-PCRs and nested PCR after 35 cycles of RT-PCR). kb, kilobases.

Southern Blot Analysis—Genomic DNA analyses were performed with several animal DNAs. Zoo blotting showed that all mammalian and avian species have the CL-L1 gene, but it is absent in yeast (Fig. 3). Slightly high expression levels of CL-L1 mRNAs were found in liver, placenta, adrenal gland, lung, small intestine, and prostate (Fig. 2B).

Localization of the CL-L1 Gene—The CL-L1 gene was localized to chromosome 8q23-24.1 by fluorescence in situ hybridization (Fig. 4). In 25 of 25 metaphase preparations, hybridiza-

**Fig. 2. Detection of CL-L1 mRNA by Northern blot and RT-PCR analyses of poly(A)* and total RNAs from various human tissues.** A, for Northern blot analysis, poly(A)* RNAs (2 µg) from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas were subjected to 1% agarose gel electrophoresis. Calculated sizes of detected RNAs are indicated by arrowheads. B, RT-PCR analyses using total RNAs (1 µg) from brain (lane 1), heart (lane 2), kidney (lane 3), liver (lane 4), lung (lane 5), trachea (lane 6), bone marrow (lane 7), colon (lane 8), small intestine (lane 9), spleen (lane 10), stomach (lane 11), thymus (lane 12), mammary gland (lane 13), prostate (lane 14), skeletal muscle (lane 15), testis (lane 16), uterus (lane 17), placenta (lane 18), adrenal gland (lane 19), pancreas (lane 20), salivary gland (lane 21), and thyroid (lane 22) were carried out (28 or 35 cycles of RT-PCRs and nested PCR after 35 cycles of RT-PCR). kb, kilobases.

**Fig. 3. Zoo blotting of genomic DNAs from animals and yeast probed with CL-L1 cDNA.** The blot contains EcoRI-digested genomic DNAs from human, rhesus monkey, cow, dog, rabbit, rat, mouse, chicken, and yeast (S. cerevisiae). kbp, kilobase pairs.

**Fig. 4. Localization of the CL-L1 Gene by fluorescence in situ hybridization.** The CL-L1 gene was localized to chromosome 8q23-24.1 by fluorescence in situ hybridization (Fig. 4). In 25 of 25 metaphase preparations, hybridiza-

CL-L1 is a single copy gene (data not shown).
tion signals were observed to the long arm of chromosome 8 in band q23-q24.1. In 22 samples, both copies of chromosome 8 were labeled, and in three samples, a signal was detected on one copy of chromosome 8. Its position was confirmed by PCR analysis using DNA from human monochromosomal hybrid cells (data not shown).

Characterization of Recombinant CL-L1 Sequences of the Neck and CRD Domains in E. coli—Although CL-L1 has collectin organizations that predict mannose or N-acetylglucosamine binding, we sought to verify this lectin activity using recombinant CL-L1 CRDs. Previously, we made collectins lacking the collagen domain in E. coli and characterized their biological activities (19, 31). In this experiment, we made two recombinant CL-L1 CRD fusion proteins: CL-L1 CRD-histidine tag fusion protein (CL-L1-CDRhis) and CL-L1 CRD-maltose binding fusion protein (CL-L1-CRDmal). After solubilization, the proteins were applied to mannan or maltose columns, but they did not bind (data not shown). Both recombinant CL-L1 fusion proteins were purified by tag ligands to be used for sugar-blot assays; only CL-L1-CRDmal was used for ELISA because CL-L1-CRDhis is insoluble in TBS. SDS-PAGE showed that the fusion proteins have different molecular sizes of 22 kDa (CL-L1-CRDhis) and 60 kDa (CL-L1-CRDmal) (Fig. 5).

Both proteins were immunostained by rabbit polyclonal anti-CL-L1-CRDhis serum (see Fig. 8A). Sugar-blot analyses showed that the two recombinant CL-L1 CRDs from E. coli and recombinant human MBP from Chinese hamster ovary cells were stained; only maltose-binding protein used as a negative control was not stained (Fig. 5). CL-L1-CRDhis was stained more strongly than CL-L1-CRDmal and human MBP. Mannan (100 μg/ml) and 10 mM EDTA inhibited the collectins from binding to the α-β-mannose BP-probe.

On the other hand, ELISA analyses showed that the α-β-mannose BP-probe bound to CL-L1-CRDmal coated on 96-well microwells at its high concentration (Fig. 6). EDTA (10 mM) and mannan (10 mg/ml) inhibited MBP from binding to the α-β-mannose BP-probe completely, but inhibited CL-L1-CRDmal binding only slightly. EDTA inhibited more strongly than mannan. A comparison of saccharide specificities by ELISA showed that CL-L1 has affinity for mannan, fucose, and galactose; a lower affinity for N-acetylglucosamine; and the lowest affinity for α-β-mannose BP-probe.

Expression of CL-L1 in Human Tissues—To examine the expression of CL-L1 at the protein level, we performed immunoblot analyses of cytosolic and microsomal fractions from liver using rabbit anti-CL-L1-CRDhis serum. CL-L1 was detected only in the cytosol, but not in microsomes (Fig. 8A) or mitochondria or extracts of nuclei (data not shown). On the other hand, MBP existed in the cytosol and microsomes. This antisera reacted with CL-L1-CRDhis and CL-L1-CRDmal, but not with MBP. The antisera detected a band corresponding to ~40 kDa in liver. The immunofluorescence analyses in human primary hepatocyte cells showed that CL-L1 was expressed in the cytoplasm, as was MBP (Fig. 8B). Its staining was inhibited by addition of another recombinant CL-L1-CRDmal fusion pro-
tein. Immunoblotting of cell culture medium from hepatocyte cells showed only the band of MBP, but no specific bands of CL-L1.

Sequence Alignment with Collectins from Other Animal Species—To compare the amino acid sequences of CL-L1, MBPs, SP-D, conglutinin, and SP-A, the sequences were aligned with that of CL-L1 (Fig. 9A). This new collectin has the four major domains: an N-terminal cysteine-rich domain, a collagen-like domain, a neck domain, and a carbohydrate recognition domain (Fig. 1). It is composed of 277 amino acids, whereas human, rabbit, and bovine MBPs are composed of 248, 247, and 249 amino acids, respectively. Collectins usually have two or more cysteines in their N-terminal domains that are conserved in all species and are involved in oligomerization. However, this new collectin has only a single cysteine in its N-terminal domain. A collagen domain of 24 Gly-X-Y amino acid repeats is found in CL-L1, without interruption at the eighth repeat, whereas this interruption is conserved in most MBPs. The collagen domain also has many prolines (five residues) and lysines (12 residues) for hydroxylation, like other collectins. The neck region is a variable domain that has hydrophobic amino acids, causing the triple helical structure (32, 33). The four cysteine residues and 14 amino acid residues that form the CRD frame in CL-L1 are conserved and found in all collectins (Fig. 9A). The four repeated lysine residues constitute the most characteristic motif that is not found in any other protein examined to date. Analyses using the DNASIS Sequence Analysis Program show that CL-L1 has 29% homology to human MBP (23) when 10 gaps are allowed in the alignment.

DISCUSSION

We have been interested in studying the function and structure of collectins and their role in the immune system. Much recent data suggest that collectins play an important role in innate immunity (16). The isolation and functional characterization of novel collectins in addition to MBP, SP-D, and SP-A might provide further insights on the functions of these collectins. We screened the human EST data base for cDNA fragments that showed sequence homology to most of the collectins in their carboxyl-terminal amino acid residues and identified a cDNA fragment encoding CL-L1. Analyses of the cDNA encoding CL-L1 suggest that CL-L1 has the same domain organiza-
tion as collectins, namely an N-terminal cysteine region, a collagen-like domain, a neck domain, and a CRD (34). Furthermore, a comparison of the amino acid sequences of the CL-L1 CRD with those of collectins suggests that CL-L1 has a basic frame of CRD (four cysteines and 14 amino acid residues). The four C-terminal repeated lysine residues constitute the most characteristic motif in this collectin and are not found in other collectins or any other proteins examined to date. The phylogenetic relationship between CL-L1 and other collectins suggests that CL-L1 may belong to a novel group in the collectin family. The CL-L1 gene was located on chromosome 8q23-q24.1. Other collectins are located on chromosome 10q (35), and we are very interested in the genomic localization of CL-L1.

The genomic organization of the collectin gene family, which includes the MBP, SP-A, and SP-D groups, shows differences. The CRD and neck domains are encoded by a single exon in all collectins (34), whereas the collagen domain is encoded by two (MBP and SP-A) or five (SP-D) exons. Preliminary data on the genomic organization of CL-L1 indicate that the CRD and neck domains are also encoded by a single exon, but the collagen-like domain is encoded by five exons (data not shown), and the number of amino acids in each exon is different from that in other collectins. These data and chromosome mapping results indicate that this new collectin developed differently than other collectins. More detailed genomic studies of its organization are needed.

Northern and Western blot analyses indicated that CL-L1 is expressed in ubiquitous organs (mainly expressed in liver, placentas, and adrenal gland), whereas SP-D and SP-A are expressed in lung and gastrointestinal tracts, and RT-PCR studies show that MBP is expressed in murine kidney and liver. RNA blotting showed that CL-L1 is expressed only faintly in placentas, and Western blotting of placenta showed bands similar to those in liver, but in trace amounts (data not shown). RT-PCRs and nested PCR after RT-PCR showed more sensitive results. CL-L1 gene expression is found in most tissues, except skeletal muscle. The expression level is varied in individual tissues. The high expression organs are liver, placenta, and

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**Fig. 9. Alignments and phylogenetic tree of CL-L1 and several collectins.** A, the amino acid sequences of CL-L1, human MBP, rabbit MBP, bovine MBP, rhesus MBP-A and MBP-C, mouse MBP-A and MBP-C, rat MBP-A and MBP-C, human SP-A, rat SP-D, bovine SP-D, bovine CL-43, and bovine conglutinin were aligned to look for comparisons. Shaded residues are identical. Dashes indicate gaps included for better alignment of the sequences to obtain maximal matching. B, the phylogenetic relationships of collectins were determined by the neighbor-joining method using amino acid sequences of CRD fragments of CL-L1, human MBP (hMBP), rhesus MBP-C (rheMBP-C), bovine MBP (bMBP), mouse MBP-A (mMBP-A), rat MBP-A, rhesus MBP-A (rheMBP-A), mouse MBP-C (mMBP-C), rat MBP-C, bovine conglutinin (bKg), bovine SP-D (hSP-D), rat SP-D, and human SP-A (hSP-A).
adrenal gland. This ubiquitous expression pattern is different from that of other collectins and galectins.

Western blot and immunofluorescence analyses indicated that CL-L1 protein is localized in cytosolic fractions from liver. Usually, collectins are secreted into the extracellular space through the endoplasmic reticulum pathway. Using anti-MBP serum with the same sample blot shows that MBP is found mainly in microsomal fractions and less in cytosolic fractions. The secreted collectins are considered to play an important role in innate immunity against pathogens invading from outside the organism (16). CL-L1 may react with internal ligands in contrast to other collectins.

Western blotting showed that MBP has molecular mass of 32 kDa, whereas its calculated molecular mass is 24.5 kDa. Other collectins showed slightly larger molecular masses than those estimated. These results indicate that CL-L1 of ~40 kDa on SDS-PAGE is not inconsistent. The amino acid residues in the neck domain of CL-L1 can form α-helices like other collectins (34). The α-helical bundle is very stable against denaturation by heat (Tm > 55 °C) or pH (pH 3.0–8.5), indicating that CL-L1 might maintain dimer and trimer structures under the reducing conditions of the cytoplasm, like other collectins.

The expression studies using CL-L1-CRDs and CL-L1-CRDmal indicated that lectin activity is preserved in CL-L1, but it is very weak. Two analyses (ELISA and sugar-blot) of weak lectin activity suggested that CL-L1 can bind to mannose at high concentrations and that this can be inhibited by mannan and EDTA. The carbohydrate-binding specificities of most collectins are for mannose-type saccharides, and saccharide specificities of the CL-L1 CRD include galactose as well as mannose, fucose, and N-acetylgalactosamine. Previously, the recombinant collectin fusion proteins produced in E. coli were used in the analysis of carbohydrate-binding specificities in recombinant collectins. All of these lectin fusion proteins attached to saccharide columns due to their high affinity. However, CL-L1-CRDmal cannot bind to saccharide columns under any buffer conditions. CL-L1-CRDmal at a high concentration (10 μM/ml) has binding activity. At such a high concentration, the maltose fusion core protein itself exhibits weak affinity for sugar BP-probes. Therefore, we understand that the difference between the binding activity of recombinant CL-L1-CRDmal and that of the maltose fusion core protein would reveal the real lectin activity of CL-L1.

The two amino acid residues of the five in collectins responsible for complexing the calcium ion involved in carbohydrate binding, namely Glu-185 and Asn-187 (36), when changed to aspartic acid and serine respectively, showed slightly larger molecular masses than those estimated. These results indicate that CL-L1 of ~40 kDa on SDS-PAGE is not inconsistent. Using anti-MBP serum with the same sample blot shows that MBP is found mainly in microsomal fractions and less in cytosolic fractions. The secreted collectins are considered to play an important role in innate immunity against pathogens invading from outside the organism (16). CL-L1 may react with internal ligands in contrast to other collectins.

The expression studies using CL-L1-CRDs and CL-L1-CRDmal indicated that lectin activity is preserved in CL-L1, but it is very weak. Two analyses (ELISA and sugar-blot) of weak lectin activity suggested that CL-L1 can bind to mannose at high concentrations and that this can be inhibited by mannan and EDTA. The carbohydrate-binding specificities of most collectins are for mannose-type saccharides, and saccharide specificities of the CL-L1 CRD include galactose as well as mannose, fucose, and N-acetylgalactosamine. Previously, the recombinant collectin fusion proteins produced in E. coli were used in the analysis of carbohydrate-binding specificities in recombinant collectins. All of these lectin fusion proteins attached to saccharide columns due to their high affinity. However, CL-L1-CRDmal cannot bind to saccharide columns under any buffer conditions. CL-L1-CRDmal at a high concentration (10 μM/ml) has binding activity. At such a high concentration, the maltose fusion core protein itself exhibits weak affinity for sugar BP-probes. Therefore, we understand that the difference between the binding activity of recombinant CL-L1-CRDmal and that of the maltose fusion core protein would reveal the real lectin activity of CL-L1.

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