Molecular Cloning of Mouse Collectin Liver 1

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Collectins are members of the superfamily of vertebrate C-type lectins that contain a collagen-like region, and are involved in first-line host defense. We earlier cloned and characterized a new kind of collectin, collectin liver 1 (CL-L1). In this study, we isolated the mouse homologue of CL-L1 encoding 277 amino acid residues; its deduced protein sequence was 88% identical with human CL-L1. Mouse CL-L1 mRNA was expressed mainly in the liver and stomach, but was found also in muscles, testes, intestines, and embryos. In mouse embryos, the level of CL-L1 mRNA gradually increased with embryonic age. In 16-day-old mouse embryos, CL-L1 mRNA was expressed in the liver, amnion, and visceral yolk sac. The mouse CL-L1 gene, Cll1, was found on chromosome 15 in a region syntenic with human chromosome 8q. CL-L1 was a highly conserved protein in mammals, birds, and fish.

Key words: collectin liver 1; collectin; mouse; cDNA; chromosome

Collectins are group III proteins of the vertebrate C-type lectin superfamily. They consist of four characteristic structures: an N-terminal cysteine-rich region, a collagen-like region with Gly-Xaa-Yaa repeats, a neck region, and a carbohydrate recognition domain (CRD) region. They can be divided into five subgroups on the basis of their amino acid sequences: mannan-binding proteins (MBPs) including MBP-A and MBP-C, surfactant protein A (SP-A), the surfactant protein D (SP-D) subgroup containing bovine conglutinin and collectin 43 (CL-43), collectin liver 1 (CL-L1), and collectin placenta 1 (CL-P1).

Collectins are key molecules in first-line host defense. They bind to sugars on the cell surface of a variety of microorganisms through their CRDs in a calcium-dependent way. Thereby MBP and conglutinin can act as opsonins, SP-D can aggregate bacteria, and also inhibit hemagglutination by influenza A virus as MBP and conglutinin do. SP-A can increase attachment of Pneumocystis carinii to alveolar macrophages. Only MBP can activate the complement system by the lectin pathway. Collectins defend the host from pathogenic microorganisms in vivo. In humans, low levels or the absence of MBP resulting from a mutation is associated with recurrent infections in both infants and adults. SP-A knockout mice are susceptible to group B streptococcal and respiratory syncytial virus infections.

We have identified two genes encoding polypeptides carrying four structural motifs characteristic of collectins from a human liver and placenta cDNA libraries by using an expressed sequence tag (EST) database; the polypeptides were named CL-L1 and CL-P1. CL-L1 is different from other collectins as...
Materials and Methods

Buffers and medium. Tris-buffered saline (TBS) contained 20 mM Tris-HCl and 140 mM NaCl, pH 7.4. TBS-C was TBS containing 5 mM CaCl$_2$ and TBS-TC was TBS containing 0.05% (v/v) Tween 20 and 5 mM CaCl$_2$. Sodium citrate-sodium chloride buffer (SSC) contained 15 mM sodium citrate, 150 mM NaCl, pH 7.0. *Escherichia coli* lysis buffer 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. The column buffer contained 10 mM phosphate, pH 7.2, 500 mM NaCl, 1 mM NaN$_3$, 10 mM 2-mercaptoethanol, and 1 mM EGTA. Column buffer-T was the column buffer containing 0.25% (w/v) Tween 20. *Luria-Bertani* (LB) medium contained 1% (w/v) Bacto-Tryptone, 0.5% (w/v) Bacto-yeast extract, and 1% (w/v) NaCl. The coating buffer contained 15 mM Na$_2$CO$_3$, 35 mM NaHCO$_3$, and 0.05% (w/v) NaN$_3$, pH 9.6.

Generation of a DNA probe for screening. Primers HLIF and HLIR for amplification of portions of the human CL-L1 neck region and the CRD were designed on the basis of the sequence from positions 355 to 375 and 814 to 834 of human CL-L1 cDNA, respectively (Table 1). PCR was done with a PCR digoxigenin probe synthesis kit (Roche Applied Science) and 50 ng of a cDNA clone of human CL-L1 as a template. PCR conditions were 35 cycles of denaturation for 1 min at 92°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C. The PCR-generated probe was electrophoresed on a 1% (w/v) agarose gel (Dojindo Laboratories, Kumamoto, Japan) and extracted from the gel with a Sephaglas BandPrep Kit (Amersham Biosciences) according to the manufacturer’s manual.

Screening of a mouse liver cDNA library. A phage library was screened as previously described. In brief, 1 × 10$^9$ plaques of a mouse liver λZAP cDNA library (Stratagene, La Jolla, CA) were plated with *E. coli* BB4, incubated at 37°C for 6 h, and transferred to Nylon filters (Nytran 13N; Schleicher & Schuell, Inc., Keene, NH). The filters were prehybridized in Hybri-buffe (5 × SSC, 1% blocking reagent (Roche), 0.1% N-lauroylsarcosine, and 0.02% SDS) for 1 h at 68°C, and then hybridized for 16 h at 55°C with a digoxigenin-labeled probe at the concentration of 10 ng/ml in the Hybri-buffer. The filters were washed twice for 5 min in 2 × SSC-0.1% SDS at room temperature and then twice for 15 min in 0.5 × SSC-0.1% SDS at 55°C. The hybridized probe was detected with a digoxigenin nucleic acid detection kit (Roche) in accordance with the manufacturer’s directions.

Isolation of cDNA clones encoding mouse CL-L1. The inserts from positive clones were subcloned in pBluescript SK(−) from the λZAP vector by an in vivo excision method as described by the manufacturer (Stratagene). The subclones were sequenced with a Thermo Sequenase cycle sequencing kit and an A.L.F. autossequencer (Amersham Biosciences).

To identify the N-terminal portion of mouse CL-L1, nested PCR was done with a mouse liver λZAP cDNA library as the template under the PCR conditions described above. The primers used are shown in Table 1. The reaction mixture in 50 μl consisted of 9 × 10$^7$ pfu of phage particles, LA PCR Buffer II (Mg$^{2+}$-free), 2.5 mM MgCl$_2$, 200 mM dNTPs, 2.5

### Table 1. Primers Used in PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLIF</td>
<td>5’-TGTTGATTGTGGAAGATACCCGG-3’</td>
</tr>
<tr>
<td>HLIR</td>
<td>5’-TTACTTTTCTTCTGTAGAA-3’</td>
</tr>
<tr>
<td>M13 universal</td>
<td>5’-CGACGTGGTAAAACGCAGCGCAG-3’</td>
</tr>
<tr>
<td>M13 reverse</td>
<td>5’-CAGGAAACCAGCTATGACATGATT-3’</td>
</tr>
<tr>
<td>mCL342</td>
<td>5’-TGGTACCTGTTTGGCCTTTC-3’</td>
</tr>
<tr>
<td>T3</td>
<td>5’-AAATACCCCTCACTAAAGG-3’</td>
</tr>
<tr>
<td>mCL343</td>
<td>5’-TCCAGGAATTCACCAAGCAGAC-3’</td>
</tr>
<tr>
<td>mCL344</td>
<td>5’-TGCACATAGGCGCAGTTTCG-3’</td>
</tr>
<tr>
<td>mCLL1N1</td>
<td>5’-TCACGTCATGAAATGCTTT-3’</td>
</tr>
<tr>
<td>mCLL1C1</td>
<td>5’-GGGTGACTGTGTAACATGAG-3’</td>
</tr>
<tr>
<td>mCLL1A</td>
<td>5’-TGGAAATCCGCAACAGCGTACCATCTG-3’</td>
</tr>
<tr>
<td>mCLL1-malFE</td>
<td>5’-GGAAGCCTTTTATTTTTTCTTGACAA-3’</td>
</tr>
</tbody>
</table>

described below. The human CL-L1 gene is expressed in most tissues and localized to chromosome 8q23–q24.1. CL-L1 is a cytosolic protein with weak lectin activity. These results suggest CL-L1 is involved with different biological functions from those of other collectins. However, there is little information on the character and functions of this gene. To clarify the biological function of CL-L1 through studies with knockout mice, we have cloned and characterized a mouse homologue of CL-L1 gene.
units of Takara LA Taq DNA polymerase (Takara Shuzo Co., Ltd., Kyoto, Japan), and 1 \( \mu \)M M13 universal primer and M13 reverse primer. A second PCR was done in the same buffer with the M13 reverse primer and an mCLL342 primer using the first PCR product as the template. A third PCR was done in the same buffer with T3 primer and mCLL343 primer or mCLL344 primer with products of the second PCR as the template. Final PCR products were subcloned into pT7Blue T-vector (Novagen, Inc., Madison, WI) and sequenced as described above.

To construct full-length mouse CL-L1 cDNA, reverse transcription (RT) PCR was done with a RNA LA PCR kit (avian myeloblastosis virus) Ver. 1.1 (Takara Shuzo). The first-strand cDNA was synthesized with reverse transcriptase (avian myeloblastosis virus) Ver. 1.1 (Takara Shuzo). The first-strand cDNA was synthesized with reverse transcriptase (avian myeloblastosis virus) using an oligo dT-adaptor primer and 1 \( \mu \)g of total RNA from the liver of an adult mouse (C57BL/6J, 8 weeks old) at 42°C for 30 min. To amplify the full-length cDNA, PCR was done with RT reaction products, Takara LA Taq polymerase (2.5 units) and the primers mCLL1N1 and mCLL1C1. The PCR program was 2 min at 94°C followed by 38 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1.5 min. The PCR product was subcloned into pT7Blue T-vector (Novagen), resulting in the clone mCLL1-N1C1-9. The sequence was confirmed in both directions.

**mRNA expression of mouse CL-L1.** To identify the tissues that express mouse CL-L1 mRNA, a mouse Rapid Scan Gene Expression Panel (OriGene Technologies, Inc., Rockville, MD) was used. This detection system is based on PCR. A 96-well PCR plate contains dried first-strand cDNAs from 24 tissues or developmental stages, serially diluted over a 4-log range. The amounts of each of the 24 cDNAs have been made uniform by correction with \( \beta \)-actin cDNA to analyze mRNA expression semiquantitatively. PCR was done with this system, Takara LA Taq polymerase (0.5 units), and the gene-specific primers mCLL1N1 and mCLL1C1 under the conditions described above.

To measure mRNA expression of CL-L1 in mouse embryos, total RNA was purified from various tissues of 16-day-old mouse (ICR) embryos with ISOGEN (Nippon Gene Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. RT-PCR was done with an RNA LA PCR kit (avian myeloblastosis virus) Ver. 1.1, and the primers mCLL1N1 and mCLL1C1 as described above.

**Interspecific mouse backcross mapping.** Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*) F1 females and C57BL/6J males as described. A total of 205 N2 mice were used to map the mouse CL-L1 gene (Cll1) locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described. All blots were prepared with Hybond-N+ nylon membrane (Amersham Biosciences). The probe, an \~ 830-bp *EcoRV* fragment of mouse CL-L1 cDNA, was labeled with \([\beta ^{32} P]dCTP\) using a nick translation labeling kit (Roche); washing was done to a final stringency of 1 × SSC containing 0.1% SDS at 65°C. Fragments of 14.0, 11.5, and 10.5 kb were detected in *BglI*-digested C57BL/6J DNA and fragments of 20.0, 11.5, and 8.0 kb were detected in *BglI*-digested *M. spretus* DNA. The presence or absence of the 20.0- and 8.0-kb *BglI* *M. spretus*-specific fragments, which cosegregated, was followed in backcross mice.

A description of the probes and restriction fragment length polymorphisms (RFLPs) for the loci linked to **Cll1** including angiopoietin (*Agpt*) and myelocytomatosis oncogene (*Myc*) has been reported previously. Recombination distances were calculated using Map Manager Ver. 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

**Computer analyses.** Nucleotide sequences were analyzed using the DNASIS sequence analysis program (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). Sequence similarities to mouse CL-L1 were searched against nucleotide sequence databases containing EST databases with the deduced amino acid sequence of mouse CL-L1 as the query sequence. The BLAST search engine (http://www.ncbi.nlm.nih.gov/BLAST) was accessed in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

**Reconstruction of mouse CL-L1 cDNA and in vitro translation.** Full-length mouse CL-L1 cDNA was amplified by PCR using the primers mCLL1A and mCLL1B with the clone mCLL1-N1C1-9 as the template. The PCR product was cleaved with the restriction endonucleases *NotI* and *XbaI* (New England Biolabs, Inc., Beverly, MA) and cloned into the expression vector pNOW/CMV-A (construction to be described elsewhere), giving pNOW-mCLL1. The sequence was confirmed in at least one direction. In *vitro* translation of mouse CL-L1 cDNA was done with a TnT coupled reticulocyte lysate system (Promega Corporation, Madison, WI) according to the manufacturer’s specifications. To label lysine residues with biotin, Transcend tRNA (a charged ep-silon-labeled biotinylated lysine-tRNA complex; Promega) was added. Translated products were subjected to SDS-PAGE on a 10–20% gradient gel under reduced conditions, transferred to a polyvinylidene difluoride membrane, and detected by streptavidin-alkaline phosphatase with a colorimetric detection
Expression of Neck-CRD Region of mouse CL-L1 in E. coli. The recombinant fusion protein with the maltose-binding protein of E. coli was expressed using the expression vector pMAL-c2 system (New England Biolabs) as previously described. Briefly, a cDNA fragment encoding two repeats of Gly-Xaa-Yaa, the neck-CRD region, was amplified by PCR with the primers mCLL1-malFE and mCLL1-malR with the clone mCLL1-N1C1-9 as the template. The PCR product was digested with EcoRI and HindIII (New England Biolabs) and cloned into the vector pMAL-c2. E. coli XLI-Blue, which carries the proper insert, was grown to an OD$_{600}$ of 0.5 in 200 ml of LB medium with 0.5% glucose. After addition of isopropyl-$\beta$-d-thiogalactopyranoside to a final concentration of 1 mM, the culture was incubated for 3 h, and the cells were harvested. The cell pellets were suspended in 10 ml of lysis buffer and lysed by sonication (15 s, 70% output, 10 times). After centrifugation at 9,000 $\times$ g for 30 min, the supernatant was applied to an amyllose resin column (New England Biolabs). The column was washed with column buffer-T and then column buffer, and the recombinant fusion protein was eluted with column buffer containing 10 mM maltose.

Characterization of recombinant CL-L1 CRD produced in E. coli. The binding of a recombinant CL-L1 CRD to mannos was measured by enzyme-linked immunosorbent assay (ELISA) with a mannos-biotin probe and avidin-biotinylated peroxidase (Vector Laboratories, Inc., Burlingame, CA). Briefly, microtiter plates were coated at 4°C overnight with recombinant CL-L1 CRD or with maltose-binding protein as a negative control (50 $\mu$g/ml in 100 $\mu$l of coating buffer). The plates were washed with TBS-TC three times after each step. After being coated, the plates were blocked with Block Ace (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) at 37°C for 1 h. After being washed, the samples were mixed with an $\alpha$-d-mannose biotinylated polymeric probe (BP-probe; Seikagaku Co., Tokyo) at various concentrations (0.01, 0.1, 1, and 10 $\mu$g/ml) with or without mannann (10 mg/ml) or EDTA (10 mm). The lectins, $\alpha$-d-mannose BP-probe, and VECTASTAIN Elite ABC reagents (Vector Labs.) were added in this order and the plates were incubated at 37°C for 1 h at each step. Finally, 100 $\mu$l of 3,3',5,5'-tetramethylbenzidine substrate solution (3,3',5,5'-tetramethylbenzidine peroxidase substrate, Dojindo Laboratories) was put into well. After addition of 100 $\mu$l of 1 M phosphoric acid, the OD$_{450}$ was read with a model 450 microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA).

Results

Isolation of mouse CL-L1 cDNA and its sequence analysis

Three incomplete clones encoding only parts of the collagen-like region, the neck region, and the CRD region were isolated by screening of the mouse liver cDNA library. To sequence the upstream region of the collagen-like region, nested PCR was done with the mouse liver cDNA library. On the basis of the obtained sequence, the gene-specific primers mCLL1N1 and mCLL1C1 were generated. Finally, RT-PCR was done with liver total RNA and the above primers. The cDNA encoded an open reading frame with 277 amino acids consisting of the N-terminal region with a cysteine residue, the collagen-like region with uninterrupted 24 Gly-Xaa-Yaa repeats, the neck region, and the CRD region (Fig. 1).

Mouse CL-L1 had 85% identity with human CL-L1 at the nucleotide level and 88% identity at the amino acid level. CL-L1 had the highest percentages of identity in the collectin family when compared with mouse and human proteins (Table 2; CL-L1, 88%; MBP-C, 60%; SP-A, 69%; and SP-D, 75%). Of the four characteristic structures of collectins, the CRD was the most highly conserved (94% identity). Surprisingly, the neck region of CL-L1 had 91% identity, much higher than that of other collectins (about 30 to 60%).
The nucleotide sequence was obtained from liver RT-PCR products. The deduced amino acid sequence is under the nucleotide sequence. Nucleotides and amino acid residues are numbered on the right. This sequence has been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession number AB016429.

Fig. 1. Nucleotide Sequence of Mouse CL-L1 cDNA and the Predicted Amino Acid Sequence.

The nucleotide sequence was obtained from liver RT-PCR products. The deduced amino acid sequence is under the nucleotide sequence. Nucleotides and amino acid residues are numbered on the right. This sequence has been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession number AB016429.

like mouse and human CL-L1. Mouse CL-L1 had four repeated lysine residues in the C-terminus of the CRD region, which sequence was characteristic of human CL-L1 (Fig. 2).

In analysis of the CRD region of mouse CL-L1, the CRD sequences of mouse and human CL-L1 and mouse collectins were aligned (Fig. 3) with rat MBP-A CRD, which has been studied in detail.31,32) Mouse and human CL-L1 CRDs contained 12 of the 14 invariant residues and 17 of the 18 highly conserved residues in the C-type CRD motif,33) and also had the conserved amino acid residues required for calcium binding in rat MBP-A CRD except for the serine residues in loop L4.

*mRNA expression of mouse CL-L1*

Mouse CL-L1 mRNA was expressed mainly in the liver and stomach, also in the muscles, testes, and
Table 2. Amino Acid Sequence Identities of Collectins between Mice and Humans

The percent identity was calculated with the DNASIS sequence analysis program.

<table>
<thead>
<tr>
<th></th>
<th>CL-L1</th>
<th>MBP-A*</th>
<th>MBP-C*</th>
<th>SP-A</th>
<th>SP-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>88%</td>
<td>58%</td>
<td>60%</td>
<td>69%</td>
<td>75%</td>
</tr>
<tr>
<td>N-terminal</td>
<td>71%</td>
<td>50%</td>
<td>56%</td>
<td>38%</td>
<td>64%</td>
</tr>
<tr>
<td>Collagen-like</td>
<td>87%</td>
<td>75%</td>
<td>74%</td>
<td>80%</td>
<td>79%</td>
</tr>
<tr>
<td>Neck</td>
<td>91%</td>
<td>31%</td>
<td>42%</td>
<td>63%</td>
<td>60%</td>
</tr>
<tr>
<td>CRD</td>
<td>94%</td>
<td>58%</td>
<td>62%</td>
<td>73%</td>
<td>76%</td>
</tr>
</tbody>
</table>

* This species of mouse has two forms of MBP, MBP-A and -C, but humans have only one form of MBP. The sequences are in Genbank-NCHI/NIH with these accession numbers: mouse MBP-A, S42292; mouse MBP-C, S42294; mouse SP-A, S48768; mouse SP-D, L40156; human CL-L1, AB002631; human MBP, X15422; human SP-A, M30838; and human SP-D, X65018.

Numbers of amino acid residues of collectins from mice and humans are in parentheses divided by a solidus.

Mouse Collectin Liver 1 embryos, and more weakly in the small intestine, in the experiment with 2.5 ng of cDNA used as the template (Fig. 4A, B). When 250 pg of cDNA was used as the template, CL-L1 mRNA was found only in the liver and stomach (data not shown). The PCR products were confirmed by Southern hybridization with the probe encoding the neck-CRD region of mouse CL-L1 (data not shown).

In embryos, mouse CL-L1 was expressed from embryonic day 8.5 to 19 (Fig. 4B). The level of the expression increased along with embryonic age. On day 12.5, in particular, much CL-L1 mRNA was found. In the mouse at embryonic day 16, the amnion and visceral yolk sac as well as the liver and whole embryo contained CL-L1 mRNA (Fig. 4C).

Chromosomal localization of the mouse CL-L1 gene

The mouse chromosomal location of Cll1 was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × Mus spretus)F1 × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 3,200 loci that are well distributed among all the autosomes as well as the X chromosome.23) C57BL/6J and M. spretus DNAs were digested with several en-
zymes and analyzed by Southern blot hybridization for informative RFLPs using a mouse Cll1 cDNA probe. The 20.0- and 8.0-kb BglI M. spretus RFLPs were used to follow the segregation of the Cll1 locus in backcross mice. Cll1 was located in the proximal region of mouse chromosome 15 linked to Agpt and Myc, which have been found in human chromosome 8q22.3-q23.4) and 8q24.12-q24.13,35) respectively. Although 134 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 5), up to 169 mice were typed for some pairs of markers.

Table 3. Amino Acid Sequence Identities of CL-L1 between Mice and Other Animals

The percent identity was calculated with the DNASIS sequence analysis program.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total corresponding region</th>
<th>N-terminal region</th>
<th>Collagen-like region</th>
<th>Neck region</th>
<th>CRD region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>88% (277)</td>
<td>71% (46)</td>
<td>87% (72)</td>
<td>91% (37)</td>
<td>94% (122)</td>
</tr>
<tr>
<td>Pig</td>
<td>80% (162)</td>
<td>60% (46)</td>
<td>69% (72)</td>
<td>83% (37)</td>
<td>62% (37)</td>
</tr>
<tr>
<td>Chicken</td>
<td>65% (222)</td>
<td>39% (46)</td>
<td>56% (72)</td>
<td>62% (37)</td>
<td></td>
</tr>
<tr>
<td>Zebrafish</td>
<td>50% (165)</td>
<td>34% (46)</td>
<td>56% (72)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers of amino acid residues are in parentheses.

*The amino acid sequence data for CL-L1 of pigs, chickens, and zebrafish were obtained from EST clones.

Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere-Agpt-20/169-Cll1-10/149-Myc. The recombination frequencies (expressed as genetic distances in centimorgans, cM, ± the standard error) are -Agpt-11.8 ± 2.5-Cll1-6.7 ± 2.1-Myc.

We have compared our interspecific map of chromosome 15 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database maintained at the Jackson Laboratory, Bar Harbor, ME). Cll1 mapped in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in this locus (data not shown).

In vitro translation of mouse CL-L1 cDNA

Mouse CL-L1 was expressed by an in vitro transcription and translation coupled system (Fig. 6). This in vitro system that uses the full-length cDNA of mouse CL-L1 generated a protein product with a molecular mass of about 35 kDa, slightly larger than the predicted molecular mass of 30.5 kDa.
Characterization of recombinant mouse CL-L1 CRD produced in E. coli

Recombinant mouse CL-L1, consisting of two repeats of Gly-Xaa-Yaa, the neck-CRD region, was expressed as a fusion protein with the maltose-binding protein of E. coli. SDS-PAGE showed that the fusion protein had the molecular mass of approximately 60 kDa (data not shown). The α-D-mannose BP-probe bound to the recombinant mouse CL-L1 CRD coated on 96-well microwells at a high concentration, but binding to maltose-binding protein was slight (Fig. 7). Mannan (10 mg/ml) inhibited the probe from binding to the recombinant CL-L1 CRD, but EDTA (10 mM) did not.

Discussion

We earlier characterized a new kind of collectin, termed CL-L1.8) Here, we cloned and sequenced a mouse homologue of human CL-L1. CL-L1 was conserved between humans and mice with high amino acid identity. In particular, the neck region and CRD region had more than 90% identity of the four collectin motifs. This high degree of conservation of an amino acid sequence has never been observed for other collectins, so we suggest that CL-L1 is conserved in evolution and probably has an important function. This supposition is supported by our findings that pig, chicken, and zebrafish had EST clones encoding part of CL-L1. They had higher amino acid identities with the mouse species examined than the identity between MBPs,30) found only in vertebrate species (from mammals to fish) among ever known collectins.30,36,37)

The CRD region of mouse CL-L1 had a high degree of sequence conservation in four collectin motifs (94% similarity with that of human CL-L1) and conserved most of residues consisting of C-type CRD.
motifs as well as human CL-L1. Despite the conserved sequence of the CRD region mentioned above, ELISA with the saccharide-biotin probe showed that recombinant mouse CRD had calcium-independent lectin activity. This result was supported by dot blotting (data not shown). In rat MBP-A CRD, changes in amino acid residues in the interior of the hydrophobic core characteristic of C-type CRD change the affinity for calcium ion.\textsuperscript{32} Human CL-L1 has a weakly calcium-dependent lectin activity when the same methods as used here were used,\textsuperscript{29} so the calcium-independent lectin activity might arise because of different amino acid residues in the hydrophobic core in mouse CL-L1 CRD compared with the human CRD.

The C-terminal four consecutive lysine residues of the CRD in human CL-L1 were found in mouse CL-L1. The four lysine residues are presumed to be the nuclear localization signal (NLS) in yeast aminoacyl-tRNA synthetases,\textsuperscript{28} and are found also in human cytoplasmic methionyl-tRNA synthetase (MRS) at the C-terminal end.\textsuperscript{39} Human MRS, which catalyzes the ligation of methionine to tRNA in protein translation, is moved to the nucleolus in response to various cellular proliferation signals.\textsuperscript{40} Although it is not known if the C-terminal four lysine residues are involved in the movement of MRS to the nucleus, these characteristic residues in CL-L1 may act as an NLS, translocating CL-L1 to the nucleus. Human CL-L1 is a cytosolic protein, as found by immunofluorescence analysis,\textsuperscript{29} so if CL-L1 moves from the cytoplasm to the nucleus, signals may be needed for such translocation. Therefore, the functional role of CL-L1 with C-terminal four lysine residues is of interest.

Mouse CL-L1 mRNA was expressed in embryos and the expression level increased with embryonic aging and dramatically on day 12.5. In mouse embryos on day 8.5, the mesenchyme is abundant and blood vessels and early heart parts can be identified, but none of the organs with mouse CL-L1 mRNA in the adult have appeared. Organogenesis is greatly accelerated by day 9,\textsuperscript{41} so CL-L1 mRNA may be expressed in endoderm cells that differentiate into liver cells or other undifferentiated cells of an early stage of embryonic development. The liver begins to form by day 9.5, becomes lobular on day 11, and enlarges considerably by day 13. The stomach begins to differentiate by day 10, and expands and changes its orientation to an oblique one on day 11.\textsuperscript{41} Hence, the dramatic increase in mRNA expression of CL-L1 on day 12.5 may be related to the development of the liver, stomach, or both.

The visceral yolk sac absorbs substances from the maternal circulation, and synthesizes and secretes some serum proteins such as apolipoproteins and α1-antitrypsin.\textsuperscript{42} In addition, it is the initial site of hematopoiesis. The amnion synthesizes numerous proteins. Human SP-A and SP-D have been found in the amniotic epithelium and in amniotic fluid by immunohistochemistry.\textsuperscript{43} Therefore, the finding of CL-L1 mRNA in extraembryonic membranes (the amnion and visceral yolk sac) as well as in the embryonic liver suggests that CL-L1 is involved in mouse embryonic

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**Fig. 6.** Translation of Mouse CL-L1 cDNA in Vitro.

Full-length mouse CL-L1 cDNA was translated in vitro with a TnT coupled reticulocyte lysate system (Lane 2). The proteins labelled with biotin were subjected to SDS-PAGE on a 10–20% gradient gel under reduced conditions, transferred to a polyvinylidene difluoride membrane, and detected by streptavidin-alkaline phosphatase with colorimetric detection. Lane 1, molecular mass markers; 2, translated product with a molecular mass of about 35 kDa as indicated by the arrow.

**Fig. 7.** Binding of Recombinant Mouse CL-L1 CRD to the Mannose-biotin Probe.

Plates were coated with 50 μg/ml of mouse CL-L1 CRD (○) or maltose-binding protein (▲). After blocking, the α-D-mannose BP-probe was added at various concentrations (0.01, 0.1, 1, and 10 μg/ml) with or without mannan (10 mg/ml) or EDTA (10 mM). The binding of the proteins to the sugar-probe was measured.
development. The visceral yolk sac is derived from visceral endoderm and mesoderm. Visceral endoderm cells originate from the primitive endoderm, which does not contribute to the embryonic endoderm, and perform absorption and secretion in the function of visceral yolk sac. Visceral mesoderm cells play a role in hematopoiesis in visceral yolk sac. Therefore, it would be of interest to examine what kind of cells express CL-L1 mRNA and how such mRNA is distributed during mouse development; CL-L1 mRNA was expressed from the early embryonic stage and its function of CL-L1 is not clear.

The Cll1 locus was mapped on chromosome 15, which shares a region of homology with human chromosome 8q, consistent with the assignment of the human homologue of Cll1 to 8q23–q24.1. In mammals, the genes for SP-A and SP-D have been mapped to a region of mouse and porcine chromosome 14 syntenic with human chromosome 10, respectively. Therefore, we hypothesize that the CL-L1 gene of species other than humans may be in the region syntenic to human chromosome 8.

Our findings showed that CL-L1 is highly conserved in mammals, birds, and fish and that its gene in a mouse species is expressed in the early stage of embryogenesis when organogenesis starts. However, the biological function of the protein is unknown. Isolation of mouse CL-L1 cDNA will enable us to understand the function of this protein through the construction and analysis of mouse strains lacking Cll1. Now, generation of Cll1 knockout mice by gene targeting techniques is in progress in our groups.

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References

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