International Immunology, Vol. 13, No. 10, pp. 1283–1290

Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN

Chae Gyu Park[1], **Kazuhiko Takahara**[2], **Eiji Umemoto**[2], **Yusuke Yashima**[2],
Kazumi Matsubara[3], **Yoichi Matsuda**[3], **Bjoern E. Clausen**[1,4], **Kayo Inaba**[2] and
Ralph M. Steinman[1]

[1]Laboratory of Cellular Physiology and Immunology, Box 176, The Rockefeller University,
1230 York Avenue, New York, NY 10021-6399, USA
[2]Laboratory of Immunobiology, Department of Animal Development and Physiology, Division of Systemic
Life Science, Graduate School of Biostudies, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku,
Kyoto 606-8502, Japan
[3]Laboratory of Cytogenetics, Division of Bioscience, Graduate School of Environmental Earth Science,
and Chromosome Research Unit, Faculty of Science, Hokkaido University, Nishi 8, Kita 10, Kita-ku,
Sapporo 060-0810, Japan

[4]Present address: Department of Cell Biology and Histology, Academic Medical Center, University of
Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, Netherlands

Keywords: cell-surface molecules, chromosomal mapping, DC-SIGN, dendritic cells, gene cloning

Abstract

DC-SIGN, a human C-type lectin, is expressed on the surface of dendritic cells (DC), while a closely related human gene, DC-SIGNR or L-SIGN, is found on sinusoidal endothelial cells of liver and lymph node. Both DC-SIGN and DC-SIGNR/L-SIGN can bind ICAM-3 and HIV gp120, and transmit HIV to susceptible cells in *trans*. Here, we report the cloning of five mouse genes homologous to human DC-SIGN and DC-SIGNR/L-SIGN. Only one gene, named mouse DC-SIGN, is highly expressed in DC, and is not found in a panel of mouse macrophage and lymphocyte cell lines. The other four genes, named mouse SIGNR1 (SIGN-Related gene 1), SIGNR2, SIGNR3 and SIGNR4, are expressed at lower levels in various cells according to RT-PCR and Northern blot analyses on RNA. All the genes of mouse DC-SIGN and SIGNRs map to adjacent regions of chromosome 8 A1.2–1.3. However, like human DC-SIGN, only the mouse DC-SIGN gene is closely juxtaposed to the CD23 gene, while the other four SIGNR genes are located close to each other in a neighboring region. mRNAs of mouse DC-SIGN and three SIGNR genes encode type II transmembrane proteins (DC-SIGN, 238 amino acids; SIGNR1, 325 amino acids; SIGNR3, 237 amino acids; SIGNR4, 208 amino acids), but the SIGNR2 gene only encodes a carbohydrate recognition domain (CRD) without a cytosolic domain and a transmembrane domain (SIGNR2, 178 amino acids). Amino acid sequence similarities between the CRD of human DC-SIGN and the mouse homologues are 67% for DC-SIGN, 69% for SIGNR1, 65% for SIGNR2, 68% for SIGNR3 and 70% for SIGNR4 respectively. However, the membrane proximal neck domains in the mouse genes are much shorter than their counterparts in human DC-SIGN and DC-SIGNR/L-SIGN. This family of mouse C-type lectins is therefore complex, but only one of the new genes, DC-SIGN, is juxtaposed to CD23 and is expressed at high levels in DC.

Introduction

DC-SIGN was originally identified in human placenta as a C-type lectin that binds the HIV surface glycoprotein gp120 (1). Later DC-SIGN was re-cloned and found to be expressed mainly on the surface of human dendritic cells (DC), to bind ICAM-3 as well as HIV-1 and to transmit HIV-1 to susceptible cells in *trans* (2,3). Meanwhile, screening of cDNA clones encoding type II transmembrane proteins resulted in the identification of a partial cDNA clone with a high sequence

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The first two authors contributed equally to this work

Correspondence to: R. M. Steinman

Transmitting editor: G. Trinchieri

Received 13 June 2001, *accepted* 8 July 2001

similarity to human DC-SIGN (4). Recently its full-length cDNA was determined (5). This second human DC-SIGN homologue, named DC-SIGNR or L-SIGN, was also shown to bind ICAM-3 and HIV gp120, and to transmit HIV to susceptible cells in *trans*, like DC-SIGN (6,7). Unlike DC-SIGN, DC-SIGNR/L-SIGN was expressed at a very low level in DC, but was highly expressed in sinusoidal endothelial cells of liver and lymph node. The gene for human DC-SIGN was located adjacent to that of CD23 on chromosome 19p13 (5). The gene for human DC-SIGNR/L-SIGN was also located next to that of human DC-SIGN (5,6).

In this report, we identified five mouse homologues of human DC-SIGN. Based on similarity searches in the GenBank expressed sequence tag (EST) database and on the high-throughput genomic sequencing phase (HTGS) database, we characterized a family of mouse DNA clones homologous to human DC-SIGN. Then, we cloned the full-length cDNAs of five mouse DC-SIGN homologues by rapid amplification of cDNA ends (RACE)-PCR. However only one of the five mouse homologues, named mouse DC-SIGN, was highly expressed in spleen DC, but the other four, named SIGN-Related genes (SIGNRs), were not. Like human DC-SIGN, the mouse DC-SIGN gene was located adjacent to the CD23 gene, whilst the other four SIGNR genes were located close to each other in a neighboring region of chromosome 8 A1.2–1.3.

Methods

Mice

C57BL/6 and (BALB/c×DBA/2) F_1 mice were purchased from Taconic (Germantown, NY) or Japan SLC (Hamamatsu, Shizuoka, Japan). Mice were kept under specific pathogen-free conditions until use at 8–10 weeks of age. All experiments were conducted according to institutional guidelines.

Cell and RNA preparation

Enriched populations of splenic DC were prepared by digestion with collagenase and then selection for low-density cells via centrifugation on a Nycodenz column (14.5% w/v in PBS with 5 mM EDTA; Nycomed, Oslo, Norway) for 15 min at 4°C. Then, $CD11c^+$ DC were further enriched by one-step MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) sorting with FITC-conjugated anti-mouse CD11c and anti-FITC MicroBeads. T cells were prepared by magnetic bead depletion (Dynal, Oslo, Norway) of MHC class II, B220, NK1.1 and F4/80 positive cells. Naive B cells were prepared as $CD19^+$ cells from $CD43^-$ spleen cell suspension by two-step MACS (Miltenyi Biotec) sorting with respective mAb. For stimulation, purified B cells were cultured for an additional 2 days with goat $F(ab')_2$ anti-mouse IgM antibody (10 μg/ml; Cappel, West Chester, PA) and IL-4 (10 U/ml; R & D Systems, Minneapolis, MN) or lipopolysaccharide (LPS; 25 μg/ml, *Escherichia coli* 0111:B4; Sigma-Aldrich, St Louis, MO) respectively. Mouse cell lines of EL4, D10.G4.1, A20, WEHI 213, WEHI 279, ABE-8.1/2, P815 and P388D1 were harvested after culture in RPMI 1640 with 10% FCS, 100 U/ml penicillin G and 100 μg/ml streptomycin. Total RNA samples were isolated from the cells using an RNeasy mini kit (Qiagen, Valencia, CA).

Cloning of mDC-SIGN and mSIGNR cDNAs

A series of RACE-PCR was performed in order to clone the full-length cDNAs of mouse DC-SIGN and SIGNRs. All of the 5′-RACE-PCRs and 3′-RACE-PCRs were performed using the total RNA sample prepared from spleen $CD11c^+$ DC of C57BL/6 mice. The gene-specific primers used for 5′-RACE-PCR and 3′-RACE-PCR of mDC-SIGN and mSIGNRs are as below: mDC-SIGN-5′-RACE primer (5′-TAGGA GGAAT GTCCA GTCCC AGGGG CAGAG T-3′), mDC-SIGN-3′-RACE primer (5′-CAAGA ACTGA CCCAG TTGAA GGCTG GCGTA-3′), mSIGNR1-5′-RACE primer (5′-CCTGG ACGTA AGCTC ATCTG TCAGC TGGGT CA-3′), mSIGNR1-3′-RACE primer (5′-GGATG ATTCT AAGCA GGAGA AGATC TACCA ACAG-3′), mSIGNR2-5′-RACE primer (5′-GAAGT CTGCT GCAGG AAGCT CTGTT CATCA TCACT TTTGA-3′), mSIGNR2-3′-RACE primer (5′-TGCCT GCAGA AAATT GGATG CCCAA CTAGT GG-3′), mSIGNR3-5′-RACE primer (5′-GCCTT CAGTT GCATG AGCTC CTGGT AG-3′), mSIGNR3-3′-RACE primer (5′-GGATG GCTCA CCTCT GTCAC CCAGC TTTAC A-3′), mSIGNR4-5′-RACE primer (5′-CTTTG CAGGC AGTCA TGGAG TCATG CCAAT-3′) and mSIGNR4-3′-RACE primer (5′-ACTCT GTCGC CTCTG CCCTT GGGAT TGGAC GT-3′). RT- and RACE-PCR were performed with a SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA) following the conditions suggested by the manufacturer's manual, except for the 5′-RACE-PCR of mDC-SIGN where PCR cycles with low annealing temperatures (45–50°C) were employed. All the PCR products were cloned with a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and then sequenced with M13 sequencing primers at the Rockefeller University Protein/DNA Technology Center.

Chromosome preparation and in situ hybridization

The direct R-banding fluorescence *in situ* hybridization (FISH) method was used for the chromosomal assignment of mouse SIGNR1 and SIGNR3 genes on mouse chromosome. Preparation of R-banded chromosomes and FISH were performed as described by Matsuda *et al.* (8). For the probes, coding regions of mSIGNR1 (1.0 kb) and mSIGNR3 (0.7 kb) cDNA fragments were amplified using primer pairs of mSIGNR1 (down-stream 5′-AACCA TGAGT GACTC CACAG AAGC-3′ and up-stream 5′-GATGA GCTAG CCTTC AGTGC ATGG-3′) and mSIGNR3 (down-stream 5′-ATGAG TGACT CCATG GAATC AAAG-3′ and up-stream 5′-TTCAT TTGGT GGTGC ATGAT GAGG-3′). The PCR-amplified fragments of mouse SIGNR1 and SIGNR3 were used for probes after purified from agarose gel electrophoresis.

Northern blot analysis

A Multiple Tissue Northern blot, each lane of which contains 2 μg of purified $poly(A)^+$ RNA from various mouse tissues, was purchased from Clontech (Palo Alto, CA). Hybridization was performed using radioactive-labeled probes at 42°C in an ECL gold hybridization buffer solution (Amersham Pharmacia Biotech, Piscataway, NJ) with 5% blocking non-fat dry milk and 0.5 M NaCl overnight. Probe, each full-length encoding cDNA fragment of mDC-SIGN and mSIGNRs, was labeled with $[\alpha\text{-}^{32}P]$dCTP using Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech). Hybridized Northern blots were washed twice for 20 min at 42°C with a wash solution (0.4% SDS/0.5×SSC) and exposed to X-ray film for autoradiography. Each Northern blot was re-probed with a β-actin cDNA probe provided with Multiple Tissue Northern blot (Clontech).

RT-PCR

First-strand cDNA was synthesized from total RNA samples with a SuperScript II reverse transcriptase kit (Life Technologies, Rockville, MD). Synthesis of each cDNA was controlled using PCR primers to detect a housekeeping gene, DNA polymerase γ (POLG) (9) (down-stream 5′-GCACT TCCGC CTCCT GGCCC AGAAG CAGA-3′ and up-stream 5′-GCTCG GTCAA AGGAA ACATT GTGCC CCACC ACTAA-3′). PCR for mouse DC-SIGN, SIGNRs and CD23 was performed with sets of primers as below: mDC-SIGN (down-stream 5′-GCACT GAGAA GTGGC TGTGA AACAT GAGTG AT-3′ and up-stream 5′-GACAA GGATG ACAAC CAGCA GCACA GAACA AACA-3′), mSIGNR1 (down-stream 5′-CACAG AAGCC AAGAT GCAGC CTCTT AGCTC CA-3′ and up-stream 5′-CCTGG ACGTA AGCTC ATCTG TCAGC TGGGT CA-3′), mSIGNR2 (down-stream 5′-CATAC ATGCG CATGC ACACC CGCCT A-3′ and up-stream 5′-GAAGT CTGCT GCAGG AAGCT CTGTT CATCA TCACT TTTGA-3′), mSIGNR3 (down-stream 5′-GCACT AGTAA CCATG AGTGA CTCCA TGGAA TCA-3′ and up-stream 5′-GCCTT CAGTT GCATG AGCTC CTGGT A-3′), mSIGNR4 (down-stream 5′-AAAGA AATGA GGGCA CCACA GATGG GCTC-3′ and up-stream 5′-CTTTG CAGGC AGTCA TGGAG TCATG CCAAT-3′) and mCD23 (down-stream 5′-GGAAC TGCAT GCAAC ATATG TCCCA AGAAC TGGCT-3′ and up-stream 5′-GTCAG GGTTC ACTTT TTGGG GTGGG CCT-3′). SureStart Taq DNA polymerase (Stratagene, La Jolla, CA), a hot-start PCR polymerase, was used with the manufacturer's PCR buffer and conditions. All of the primer sets used for mDC-SIGN, mSIGNRs and POLG were designed to amplify the multiple exon-spanning cDNA sequences of target genes from their mRNA transcripts, and thus avoid the possibility to amplify DNA fragments from contaminating genomic DNAs. The cDNA fragments amplified from RT-PCR were all sequenced to confirm their authenticity.

Results

cDNA cloning of mouse DC-SIGN homologues

To find the mouse homologue(s) of human DC-SIGN and DC-SIGNR/L-SIGN, we conducted a series of sequence similarity searches in the GenBank EST database and HTGS database. We found three groups of overlapping EST sequences that shared significant homologies with human DC-SIGN and DC-SIGNR/L-SIGN. The first group included the EST sequences with the GenBank accession nos AA163654, AA914211, AI386429 and AA543877; the second group included AI507520; and the third group included AA510960, AI614060, AV052536 and AA920619. Based on these three different sequences from the overlapping EST sequences, we designed 5′- and 3′-RACE-PCR primers and, by RACE-PCR, successfully cloned the full-length cDNAs of three different mouse DC-SIGN homologue genes, named mouse SIGN-Related gene 1 (mSIGNR1), mSIGNR2 and mSIGNR3, respectively. Interestingly, all three mSIGNR genes were located close to each other on one HTGS genomic clone with the GenBank accession no. AC073706. Then we found a fourth mouse DC-SIGN homologue from this AC073706 HTGS sequence in the vicinity of mSIGNR genes. The full-length cDNA of this fourth mouse DC-SIGN homologue, named mSIGNR4, was also cloned after 5′- and 3′-RACE-PCRs.

Cloning of mouse DC-SIGN cDNA from spleen DC

Although all four mSIGNR cDNAs were cloned from the RNA sample prepared from mouse spleen DC, the RT-PCR products from all four mouse SIGNR mRNAs were not readily detected in mouse spleen DC, even after 40 cycles of RT-PCR (Fig. 1). Therefore, we decided to perform further 5′-RACE-PCRs using the 5′-RACE primers used in the cloning of the four mouse SIGNR cDNAs but with much lower PCR annealing temperatures than the calculated annealing temperature of the 5′-RACE primers. From the 5′-RACE-PCR with a low annealing temperature using mSIGNR1 5′-RACE primer, we cloned a fifth mouse DC-SIGN homologue. mRNA for this fifth gene was readily detected in mouse spleen DC by RT-PCR (Fig. 1) and consequently named mouse DC-SIGN (mDC-SIGN). By RT-PCR, the signals for mDC-SIGN in DC were as strong as for β-actin (data not shown).

According to RT-PCR studies with RNAs from various cells purified from mouse spleen (Fig. 1), only DC-SIGN was highly detected in spleen CD11c$^+$ DC, but much less in spleen T or B cells. Mouse SIGNR1 and SIGNR3 were slightly more abundant in DC than T cells and B cells, but was only found in DC at very low levels (only after 40 or more RT-PCR cycles). SIGNR2 was mainly detected in LPS-stimulated B cells, while SIGNR4 was not detected in the tested DC, T or B cells, even

Fig. 1. Five mouse homologues of DC-SIGN were detected by RT-PCR from various cells in mouse spleen. The number of PCR cycles for each panel is indicated. The different types of spleen cells in each lane are as follows: lane 1, CD11c$^+$ DC; lane 2, T cells; lane 3, B cells; lane 4, B cells treated with anti-IgM and IL-4; lane 5, B cells treated with LPS.

(A) DC-SIGN

1 MSDSKEMGKRQLRPLDEELLTSSHTRHSIKGFGFQTNSGFSSFTGCLVHS
51 QVPLALQVLFLAVCSVLLVVILVKVYKIPSSQEENNQMNVYQELTQLKAG
101 VDRL**CRSCPWDWTHFQGSCYFFSVAQKSWNDSATACHNVGAQLVVIKSDE**
151 **EQNFLQQTSKKRGYTWMGLIDMSKESTWYWVDGSPLTLSFMKYWSKGEPN**
201 **NLGEEDCAEFRDDGWNDTKCTNKKFWICKKLSTSCPSK** 238

(B) SIGNR1

1 MSDSTEAKMQPLSSMDDDELMVSGSRYSIKSSRLRPNSGIKCLAGCSGHS
51 QVPLVLQLLSFLFLAGLLLIILFQVSKTPNTERQKEQEKILQELTQLTDE
101 LTSRIPISQGKNESMQAKITEQLMQLKTELLSRIPIFQGQNESIQEKISE
151 QLMQLKAELLSKISSFPVKDDSKQEKIYQQLVQMKTELFRL**CRLCPWDWT**
201 **FLLGNCYFFSKSQRNWNDAVTACKEVKAQLVIINSDEEQTFLQQTSKAKG**
251 **PTWMGLSDLKKEATWLWVDGSTLSSRFQKYWNRGEPNNIGEEDCVEFAGD**
301 **GWNDSKCELKKFWICKKSATPCTEG** 325

(C) SIGNR2

1 MRMHTRLQFLKRVSNVAYSHGQEQAKKEKVYKEMTQLKSQINRL**CRPCPW**
51 **DWTVFQGNCYFFSKFQQNWNDSVNACRKLDAQLVVIKSDDEQSFLQQTSK**
101 **EKGYAWMGLSDLKHEGRWHWVDGSHLLFSFMKYWNKGEPNNEWEEDCAEF**
151 **RGDGWNDAPCTIKKYWICKKSAMSCTEK** 178

(D) SIGNR3

1 MSDSMESKTQQVVIPEDEECLMSGTRYSDISSRLQTKFGIKSLAEYTKQS
51 RNPLVLQLLSFLFLAGLLLIILILVSKVPSSEVQNKIYQELMQLKAEVHD
101 GI**CQPCARDWTFFNGSCYFFSKSQRNWHNSTTACQELGAQLVIIETDEEQ**
151 **TFLQQTSKARGPTWMGLSDMHNEATWHWVDGSPLSPSFTRYWNRGEPNNV**
201 **GDEDCAEFSGDGWNDLSCDKLLFWICKKVSTSSCTTK** 237

(E) SIGNR4

1 MRAPQMGSLGFLDKGHIPLVLQLLFLILFTGLLVAIIIQVSKMPSSEEIQ
51 WEHTKQEKMYKDLSQLKSEVDRL**CRLCPWDWTFFNGNCYFFSKSQRDWHD**
101 **SMTACKEMGAQLVIIKSHEEQSFLQQTSKKNSYTWMGLSDLNKEGEWYWL**
151 **DGSPLSDSFEKYWKKGQPNNVGGQDCVEFRDNGWNDAKCEQRKFWICKKI**
201 **ATTCLSKW** 208

(F) Alignment of CRD sequences

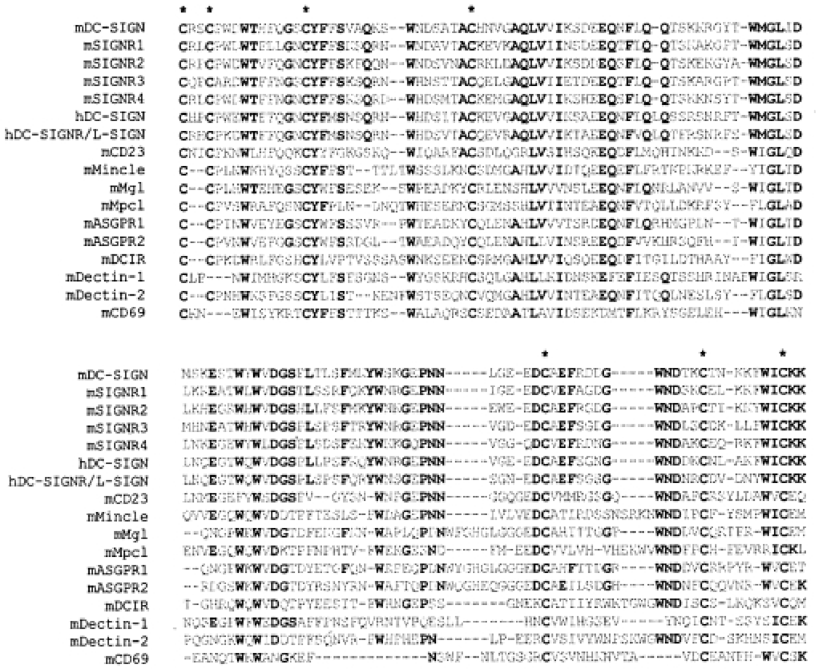

Fig. 2. Legend on facing page.

after 44 cycles of RT-PCR (Fig. 1). Interestingly, RT-PCR amplification of the SIGNR1 fragment between exon1 and exon4 revealed two dominant PCR products originating from alternatively spliced mRNAs, one of which turned out to lack exon3 that encodes the transmembrane domain (the lower molecular weight band of SIGNR1 in Fig. 1).

Structures of mouse DC-SIGN and SIGNRs

As illustrated in Fig. 2, mouse DC-SIGN (GenBank accession no. AF373408) was a 238 amino acid long type II transmembrane C-type lectin protein with a carbohydrate recognition domain (CRD), like human DC-SIGN (1) and DC-SIGNR/L-SIGN (5,6). Mouse SIGNR1 (325 amino acids, GenBank accession no. AF373409), SIGNR3 (237 amino acids, GenBank accession no. AF373411), and SIGNR4 (208 amino acids, GenBank accession no. AF373412) were also type II transmembrane C-type lectins with a CRD. However, SIGNR2 sequence (178 amino acids, GenBank accession no. AF373410) only had a CRD without a cytosolic domain and a transmembrane domain.

Even after extensive 5′-RACE-PCRs on SIGNR2 cDNA, we still could not find a SIGNR2 transcript encoding a cytosolic domain and a transmembrane domain. Therefore, the SIGNR2 cDNA sequence in our report did not appear to have originated from an unprocessed form of mRNA transcript, nor it is likely that the SIGNR2 mRNA transcript could express a functional type II transmembrane C-type lectin protein on the cell surface. Also, only the SIGNR2 cDNA transcript from 5′-RACE-PCR showed a long 5′-untranslated (5′-UT) region, ~1 kb nucleotides, in its cDNA. This long 5′-UT region, with multiple ATG initiation codon sequences, in the mRNA might hinder the efficient translation of SIGNR2 protein (10).

A major structural difference between human DC-SIGN/DC-SIGNR/L-SIGN and mouse DC-SIGN homologue proteins is the length of a neck domain, the amino acid sequences between the transmembrane domain and CRD. Both human DC-SIGN and DC-SIGNR/L-SIGN had 191 amino acid long neck domains, encoded by a single exon (5). However, mouse DC-SIGN and SIGNRs had shorter neck domains: 29 amino acids for DC-SIGN, 116 amino acids for SIGNR1, 27 amino acids for SIGNR3 and 33 amino acids for SIGNR4 (Fig. 2). The short neck domains of mouse DC-SIGN, SIGNR3 and SIGNR4 were encoded by a single exon, whereas the neck domain of SIGNR1 was encoded by four small exons, each for 29 amino acids. Although the significance of the neck domain in the function of human DC-SIGN and DC-SIGNR/L-SIGN is still unknown, our RT-PCR studies revealed that a small portion (~10%) of mouse DC-SIGN mRNA was expressed as an alternatively spliced form that lacked exon4 for encoding a neck domain (data not shown).

Even though human and mouse DC-SIGN homologue proteins were somewhat different in their overall sizes, especially in the neck domain, all the CRD of human and mouse DC-SIGN homologue proteins had a similar size and shared high sequence homologies (Fig. 2F). The amino acid sequence similarities between the CRD of human DC-SIGN and mouse homologues are 67% for DC-SIGN, 69% for SIGNR1, 65% for SIGNR2, 68% for SIGNR3 and 70% for SIGNR4 respectively.

Genomic structures of mouse DC-SIGN and SIGNR genes

As the Genome Project progresses, considerable mouse genomic sequence information has become available to the public. After a search in the GenBank HTGS database, we could locate all of the mouse DC-SIGN and SIGNR genes on several HTGS genomic clones. Based on the overlapping sequences between HTGS clones, we drew a mouse genomic map of DC-SIGN and SIGNR genes (Fig. 3). All four SIGNR genes were co-located close to each other on the same mouse HTGS clone of the GenBank accession number AC073706. However, the mouse DC-SIGN gene was located on another HTGS clones of the GenBank accession nos AC087183 and AC079491. Also, like human DC-SIGN and DC-SIGNR/L-SIGN genes, both of which were co-located with CD23 on same human genomic clones (5,6), mouse DC-SIGN gene was co-located with CD23 on both of mouse HTGS clones AC087183 and AC079491 (Fig. 3). We also determined the chromosomal assignment of SIGNR1 and SIGNR3 genes on mouse chromosome by the direct R-banding FISH method (8). The gene cluster of mouse SIGNRs, DC-SIGN and CD23 were mapped in the region of chromosome 8 A1.2–1.3 (Fig. 3).

Of the five mouse DC-SIGN homologues identified, the four genes juxtaposed on the HTGS clone AC073706 were not abundantly expressed in spleen DC, whilst the fifth co-localized with CD23 on the HTGS clones AC087183 and AC079491, and was abundantly expressed in spleen DC. Therefore, the latter was best termed mouse DC-SIGN and the other four genes SIGNRs.

Expression of DC-SIGN and SIGNRs in various tissues

Northern blot analyses of various mouse tissues revealed that mouse DC-SIGN was expressed at a relatively high level in spleen and lung, and at lower levels in kidney, heart, thymus and lymph nodes (Fig. 4; thymus and lymph nodes data not shown). SIGNR1 was expressed at a high level in lymph nodes (data not shown) and at a low level in spleen (Fig. 4). Northern blot of SIGNR1 showed two distinct bands that appeared to originate from alternatively spliced mRNAs. The alternatively spliced mRNAs of SIGNR1 was also detected from RT-PCR analysis of SIGNR1 (Fig. 1). SIGNR2

Fig. 2. Amino acid sequences of five mouse DC-SIGN homologues are shown for (A) DC-SIGN, (B) SIGNR1, (C) SIGNR2, (D) SIGNR3 and (E) SIGNR4. The transmembrane domains are double underlined, the membrane proximal neck domains are boxed and the CRD are given in bold. (F) The CRD of mouse and human DC-SIGN homologues are aligned with other C-type lectins. Conserved amino acids among mouse and human DC-SIGNs are in bold, and cysteine residues conserved in mouse and human DC-SIGNs are marked with asterisks. The GenBank accession nos are as follows: mouse DC-SIGN, AF373408; mouse SIGNR1, AF373409; mouse SIGNR2, AF373410; mouse SIGNR3, AF373411; mouse SIGNR4, AF373412; human DC-SIGN, M98457; human DC-SIGNR/L-SIGN, AF245219; mouse CD23, M99371; mouse Mincle, AB024717; mouse Mgl, AF132744; mouse Mpcl, NM_010819; mouse ASGPR1 (asialoglycoprotein receptor 1), U08372; mouse ASGPR2 (asialoglycoprotein receptor 2), NM_007493; mouse DCIR, AJ133533; mouse Dectin-1, AF262985; mouse Dectin-2, AF240357; mouse CD69, P37217.

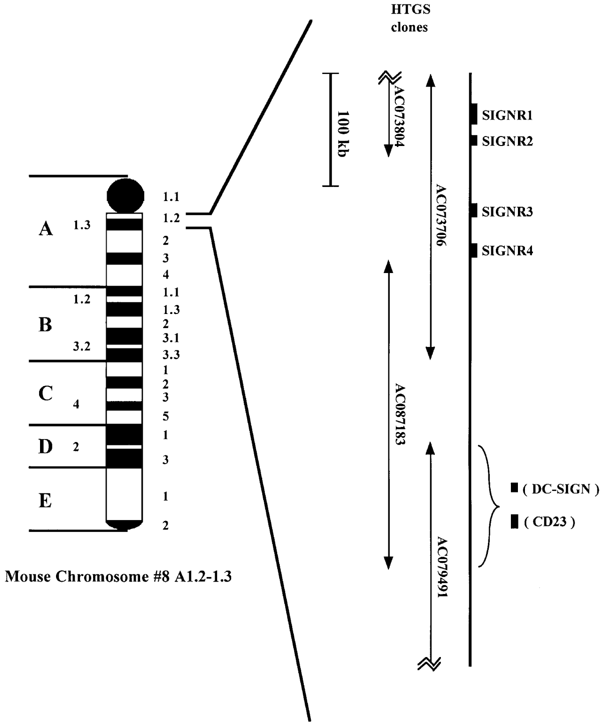

Fig. 3. Chromosomal location and genomic organization of mouse DC-SIGN homologues and CD23 are illustrated. Mouse SIGNR1 and SIGNR3 genes were physically mapped by FISH on chromosome 8 A1.2–1.3, while the contiguous genomic map of overlapping HTGS clones in the vicinity is depicted. The chromosomal direction of genomic map was not determined, and the exact locations of DC-SIGN and CD23 were not resolved, because HTGS clones AC087183 and AC079491 are currently draft sequences of unordered pieces. However, both DC-SIGN and CD23 were located close to each other in the overlapping region between HTGS clones AC087183 and AC079491.

was expressed at very low levels in most of the tissues tested with different sizes, which might originate from alternative splicing, but SIGNR2 was expressed more prominently in testis (Fig. 4). SIGNR3 was expressed at a low level in spleen and not easily detected in other tissues (Fig. 4). However, we could detect SIGNR3 at a low level in lymph nodes when a Northern blot of poly(A)$^+$ RNA was probed (data not shown). SIGNR4 was not easily detected in most of the tissues tested, but very low levels of SIGNR4 were detected in spleen as two distinct bands, suggesting a possible alternative splicing (Fig. 4). According to the cDNA size (~1.8 kb) of SIGNR4, the high mol. wt signal appeared to represent the mRNA encoding the full-length SIGNR4 protein. Interestingly, testis expressed the low mol. wt band of SIGNR4 mRNA at high levels.

RT-PCR detection of mouse DC-SIGN and CD23

We further performed RT-PCR of mDC-SIGN in various mouse cell lines (Fig. 5A). Our panel of lines included standard macrophages (P388D1), B cells (A20, WEHI 213, WEHI 279 and ABE-8.1/2), T cells (EL4 and D10.G4.1) as well as the P815 mastocytoma. None of cell lines tested had detectable mDC-SIGN, even after 40 cycles of RT-PCR.

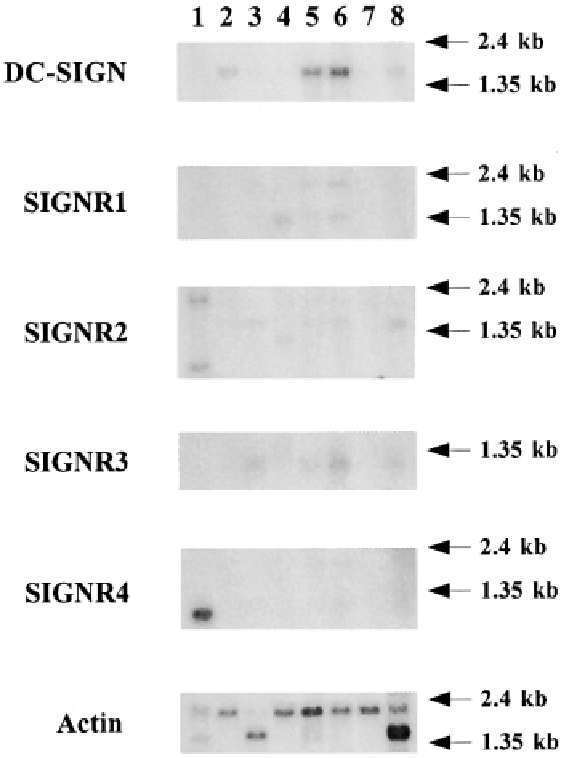

Fig. 4. Northern blot analyses of mouse DC-SIGN homologues were performed with poly(A)$^+$ RNA from various mouse tissues. RNA samples of mouse tissues are as follows: lane 1, testis; lane 2, kidney; lane 3, skeletal muscle; lane 4, liver; lane 5, lung; lane 6, spleen; lane 7, brain; lane 8, heart. Two isoforms of β-actin mRNA, a 2- and a 1.8-kb form, are present in several tissues.

Since mouse DC-SIGN was juxtaposed to CD23 on the chromosome, we examined the expression levels of CD23 in purified cells from mouse spleen by RT-PCR, in comparison with DC-SIGN (Fig. 5B). CD23 was abundantly expressed in spleen B cells and at less but significant levels in spleen CD11c$^+$ DC, but was not detectable in spleen T cells. Mouse DC-SIGN was abundantly expressed in spleen CD11c$^+$ DC (Fig. 5B), as in the prior experiment.

Discussion

The recent identification of DC-SIGN (2,3) has been important for several reasons. The gene is expressed at high levels and selectively in DC. It is the first adhesion molecule that seems to be selectively expressed on DC, being the counter-receptor for ICAM-3 on resting T cells (2). The DC-SIGN/ICAM-3 interaction may account for the capacity of DC to bind to syngeneic T cells (11), allowing the TCR to scan the DC surface for MHC–peptide ligands early in the initiation of an immune response (12). DC-SIGN proved to be identical to a previously cloned HIV-1 envelope binding lectin and indeed this lectin functions to transmit HIV-1 to T cells (3). Some complexity has emerged with the identification of an additional SIGN gene termed DC-SIGNR or L-SIGN (5,6). This gene product also binds HIV-1 but, interestingly, DC-SIGNR is

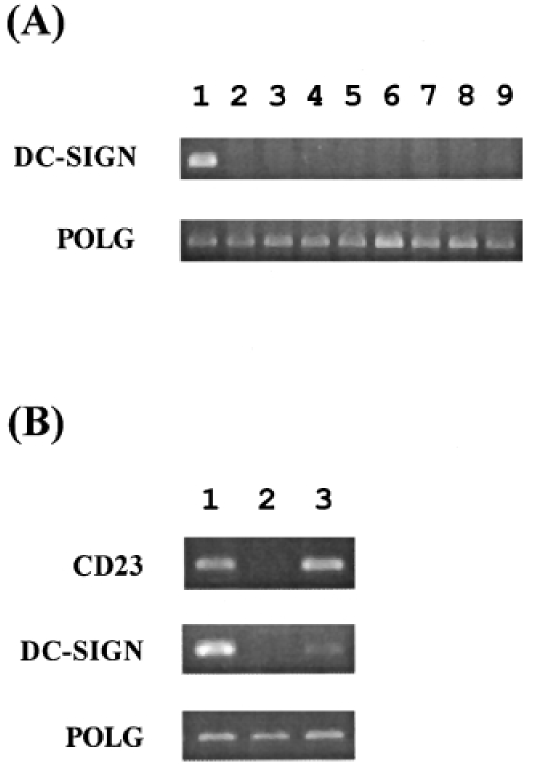

Fig. 5. (A) DC-SIGN was detected by RT-PCR from various mouse cell lines. The mouse cells in each lane are as follows: lane 1, spleen CD11c$^+$ DC; lane 2, EL4; lane 3, D10.G4.1; lane 4, A20; lane 5, WEHI 213; lane 6, WEHI 279; lane 7, ABE-8.1/2; lane 8, P815; lane 9, P388D1. (B) CD23 and DC-SIGN were detected by RT-PCR from various cells in mouse spleen. The different types of spleen cells in each lane are as follows: lane 1, CD11c$^+$ DC; lane 2, T cells; lane 3, B cells.

expressed weakly if at all in DC, and is found primarily on sinusoidal endothelial cells in liver and lymph node (6,7).

Here we have pursued the identification of mouse DC-SIGN. The EST and HTGS databases first led us to four new mouse homologues. Although we cloned the cDNAs for each gene from a splenic DC RNA preparation, none of these genes were expressed abundantly in DC and none were closely linked to CD23, as is the case with human DC-SIGN and DC-SIGNR/L-SIGN. We therefore searched further with our 5′-RACE primers, but with much lower PCR annealing temperatures. This allowed us to identify a fifth mouse gene that appears to be authentic mouse DC-SIGN. It is abundantly expressed in mouse DC with RT-PCR signals approaching that of actin and the gene is juxtaposed to CD23. The other four genes, also located on chromosome 8 A1.2–1.3 but more removed from CD23, are best named SIGNR1–4, for SIGN-Related genes.

The structure of all five mouse genes are homologous to one another, and to human DC-SIGN and DC-SIGNR/L-SIGN, in the single external C-type lectin or CRD. However, the membrane proximal neck domain is much shorter for the mouse SIGNs (27–116 amino acids) relative to human DC-SIGN and DC-SIGNR/L-SIGN (both 191 amino acids). The function of this membrane proximal neck domain remains unknown, although its sequence is distinct.

Further studies on the expression of mouse DC-SIGN are underway to retrieve antibodies and to better assess expression in different sources of DC, e.g. DC subsets and different states of maturation. The detailed tissue distribution of the other four genes, which we propose to term SIGNR1–4, also awaits additional experiments. However, SIGNR2 and SIGNR4 are unusual because of their abundance in testis. The cloning and limited characterization of the mouse SIGN homologues reveals the complexity of this new family of C-type lectins, and provides a foundation for future work on their function in cell–cell interactions and carriage of infectious agents.

Acknowledgements

We are grateful to Dr Yongwon Choi and Dr Nacksung Kim for providing us valuable reagents and equipment. We thank Mr Cheolho Cheong, Dr Shiaoching Gong, Dr Soon Gyu Hong and Dr Michel C. Nussenzweig for helpful discussions on experiments and data analysis. We appreciate Drs Susumu Ikehara and Muneo Inaba (Kansai Medical University, Moriguchi, Osaka, Japan) for helpful advice on cell preparation and for critical reading of the manuscript. This work was supported by grants from the NIH (AI13013 and AI40045) and the Ministry of Education, Science, Sports and Culture of Japan [Grant-in-Aid for Scientific Research on Priority Areas (C:12204062, A:11140232), 10770096, 11470085] through Special Coordination Funds of the Science and Technology Agency of the Japanese Government. B. E. C. was the recipient of a long-term fellowship of the Human Frontier Science Program Organization (HFSPO).

Abbreviations

CRD	carbohydrate recognition domain
DC	dendritic cell
EST	expressed sequence tag
FISH	fluorescence *in situ* hybridization
HTGS	high-throughput genomic sequencing phase
LPS	lipopolysaccharide
POLG	DNA polymerase γ
RACE	rapid amplification of cDNA ends
UT	untranslated

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