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Cutting Edge: DC-SIGN; a Related Gene, DC-SIGNR; and CD23 Form a Cluster on 19p13[1,2]

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DC-SIGN is a C-type lectin, expressed on a dendritic cell subset. It is able to bind ICAM3 and HIV gp120 in a calcium-dependent manner. Here we report the genomic organization of *DC-SIGN* and map it to chromosome 19p13 adjacent to the C-type lectin *CD23* (FcεRII). We also report a novel, closely linked gene, *DC-SIGNR*, which shows 73% identity to *DC-SIGN* at the nucleic acid level and a similar genomic organization. Proteins encoded by both genes have tracts of repeats of 23 aa, predicted to form a coiled coil neck region. They also possess motifs that are known to bind mannose in a calcium-dependent fashion. We show concomitant expression of the two genes in endometrium, placenta, and stimulated KG1 cells (phenotypically similar to monocyte-derived dendritic cells). The existence of a *DC-SIGN*-related gene calls for reinterpretation of the HIV data to consider possible DC-SIGN/DC-SIGNR hetero-oligomerization. ***The Journal of Immunology*****, 2000, 165: 2937–2942.**

DC-SIGN, originally described in 1992 as a C-type lectin able to bind the HIV surface protein, gp120 (1), has been shown to be important for efficient infection with HIV (2). The DC-SIGN molecule is used by HIV to attach to dendritic cells in the genitourinary tract and rectum (3). EGTA and mannan can inhibit this binding (2). Geitjenbeek et al. (2) suggest that dendritic cells then carry HIV particles to lymph nodes, where the infection of T lymphocytes via receptors such as CD4 and CCR5 may occur. DC-SIGN also binds the highly glycosylated molecule, ICAM3, found on T lymphocytes, enhancing the interaction of dendritic cells with T lymphocytes (3). A partial cDNA of a second, closely related gene was identified in 1999 by Yokoyama-Kobayashi et al. (4). In this study we investigated the mapping, genomic organization, and expression of *DC-SIGN* and a closely related gene, *DC-SIGNR*.

Materials and Methods

PCR and cloning of full genes and cDNAs

End primers (*DC-SIGN*-F (CTAAAGCAGGAGTTCTGGAC), *DC-SIGN*-R (CTAAAGGTCGAAGGATGGAG), *DC-SIGNR-F* (AACATCTGGGGA CAGCG), and *DC-SIGNR*-R (GCAGTTACAACATTTACCACTT)) were designed from published cDNA sequences (1, 4). Genes were amplified from genomic DNA using the Promega Taq DNA polymerase system (Promega, Southampton, U.K.), dNTPs (2.0 mM), magnesium (2.0 mM), and cycle conditions of 92°C for 1 min, 60°C for 1 min, and 72°C for 2–6 min. PCR products were cloned with a TOPO-XL cloning kit (Invitrogen, San Diego, CA). cDNAs representing the entire coding region were cloned from placental cDNA as described above. Clones were sequenced as described previously (5). Predicted protein sequences were analyzed using Pix (http://menu.hgmp.mrc.ac.uk/menu-bin/Pix/) and ExPasy (http://www.expasy.ch/).

Mapping of the genes

Mapping was conducted by PCR using a radiation hybrid panel (HGMP-MRC) (6). A 366-bp sequence-tagged site from exon 4 of *DC-SIGN* was amplified as described with primers Lizol88 (CGCGATCTACCAGAAC CTG) and Lizol91 (TCCTGGTAGATCTCCTGCAT). The map position was analyzed with the RhYme program (http://menu.hgmp.mrc.ac.uk/menu-bin/RHyME/).

P1 artificial chromosome (PAC)[4] identification

Gridded human RPCI 1–5 PAC libraries (7) were hybridized for 20 h with 50 ng of [α-^{32}P]dCTP-labeled full-length *DC-SIGN* cDNA. Membranes were washed to a stringency of 1 × SSC/0.1% SDS at 65°C and exposed to x-ray film at −70°C for 24 h (8–10). Positive clones were provided by the Human Genome Mapping Project Resource Center. DNA was extracted using standard techniques (9). PAC DNA (150 ng) was digested with *Not*I (New England Biolabs, Beverly, MA) and separated on a pulse-field gel, with ramped switch times from 1–13 s at 200 V for 16 h. PCR screening confirmed the presence of *DC-SIGN*, *DC-SIGNR*, or CD23 on the PACs. Results were confirmed by sequencing (5).

DNA blot analysis

PAC DNA (20 μg) was digested with the restriction enzymes *Eco*RI (New England Biolabs) and *Pst*I (New England Biolabs), followed by electrophoresis on 0.8% agarose-TBE gel. DNA was transferred to a Hybond N$^+$ nylon membrane. Membranes were prehybridized for 1 h in hybridization buffer (0.001 M EDTA, 0.5 M sodium phosphate (pH 7.2), and 7% SDS). Fifty nanograms of a 140-bp probe across exon 5 of *DC-SIGN* was labeled with [α-^{32}P]dCTP and hybridized for 18 h at 65°C (7). Membranes were washed to a stringency of 0.1% SSC with 0.1% SDS at 65°C and exposed to Biomax MR film (Eastman Kodak, Rochester, NY) for 12 h.

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[2] The following accession numbers have been deposited in GenBank: AF209479 (*DC-SIGN* gene), AF209480 (*DC-SIGNR* gene, exons 1 and 2), AF209481 (*DC-SIGNR* gene, exons 3–8), and AF245219 (*DC-SIGNR* cDNA).

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[4] Abbreviations used in this paper: PAC, P1 artificial chromosome; CRD, carbohydrate recognition domain.

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Cell culture and cDNA

Human YT, U937, Ind, 293T, and HCMED1-E6, were cultured in RPMI 1640, 10% FCS, 100 U/ml penicillin, and 100 U/ml streptomycin. Human Jurkat, T47D, MRC5, and C33a were cultured in DMEM, 10% FCS, 100 U/ml penicillin/streptomycin, and 2 mM L-glutamine. HT29 cells were cultured in DMEM/F12 (1/1), 10% FCS, and 100 U/ml penicillin/streptomycin. KG1 cells were cultured in IMDM, 20% FCS, 100 mM L-glutamine, and 100 U/ml penicillin/streptomycin and stimulated with PMA (10 ng/ml) and ionomycin (100 ng/ml; Sigma, St. Louis, MO) (11). Langerhans-type dendritic cells (12, 13) were donated by Paul Lehner (Cambrdige University, Cambridge, U.K.). Total RNA was isolated using th RNA-easy minikit (Qiagen, Crawley, U.K.). Placental and endometrial cDNA were donated by Amanda Evans (Cambridge University). Monocyte-derived dendritic cell cDNA (14) was donated by Jason Caulfield (Kings College, London, U.K.).

RT-PCR

First-strand cDNA was prepared using polyT and the Superscript kit (Life Technologies, Paisley, U.K.). cDNA synthesis was controlled using GAPDH primers (forward, ACCACAGTCCATGCCATCAC; reverse, TCCACCACCCTGTTGCTGTA). PCR for *DC-SIGN* and *DC-SIGNR* was performed with: *DC-SIGN*: Lizol125, TGCAACTCCTCTCCTTCAC; Lizol202, CTTTGCAGGCGGTGAT; and *DC-SIGNR*: Lizol126, TGCAACTCCTCTCCTTCAT; Lizol201, CTGGCAGGCGGTGAC. PCR conditions were 92°C for 1 min, 61°C for 1 min, and 72°C for 2 min for 35 cycles, with magnesium at 1.5 mM. PCR was controlled using *DC-SIGN* and *DC-SIGNR* cDNA clones. PCR products were sequenced (5).

Results

Cloning and sequencing of cDNAs

Using the GenBank database sequence for DC-SIGN (1) and a closely related partial cDNA sequence, AB015629 (4), end primers were designed, and full-length cDNAs were cloned from human placenta. Comparison of our full-length AB015629 clone, now termed DC-SIGNR, with the previously published sequence revealed two extra exons. Additionally a 330-bp 3′ intron (Fig. 4*A*) previously described in AB015629 cDNA had been spliced out of our cDNA.

Both DC-SIGN and DC-SIGNR DNA sequences encode type II integral membrane proteins. The two genes show 73% identity at the nucleic acid level and 77% identity at the amino acid level. Predicted protein sequences from the full-length cDNAs are compared in Fig. 1. The cytoplasmic tails of both genes contained a di-leucine motif, which is a recognized internalization sequence (15). Exon 2 of *DC-SIGN*, but not that of *DC-SIGNR*, encodes a YXXL motif, as a further potential internalization signal (16). The putative transmembrane domains of DC-SIGN and DC-SIGNR consist of approximately 18 and 22 aa, respectively. An *N*-linked glycosylation sequence is found immediately after the transmembrane domains of both molecules (17), followed by a neck, encoded by a single exon, containing seven repeats of the 23 aa sequence KAAVGELxEKSKxQEIYQELTxL. The carbohydrate recognition domains (CRDs) are encoded by three separate exons, as described for CD23 (18) and the asialoglycoprotein receptors (19). These CRDs contain all the residues previously shown to be required for calcium-dependent binding of mannose (Fig. 2) (20).

Mapping of DC-SIGN and DC-SIGNR

Mapping of DC-SIGN and DC-SIGNR was conducted by performing PCR across exon 4 of DC-SIGN on a radiation hybrid panel (HGMP) (6). Results were consistent with a localization on chromosome 19p13.3. The RPCI PAC libraries (7) were screened with a probe consisting of the full-length sequence of DC-SIGN. DC-SIGN and DC-SIGNR were found on overlapping PACs, the sizes of which were determined by pulse field gel electrophoresis (Fig. 3). PACs were screened by PCR using exon primers for DC-SIGN and DC-SIGNR as well as CD23, a C-type lectin known to map to 19p13 (21), revealing the gene order shown in Fig. 3. The smallest PAC containing DC-SIGN, DC-SIGNR, and CD23 has an insert size of 105 kbp. These data concur with preliminary high throughput genomic sequence data from The Lawrence Livermore center and with the localization of the CD23 gene (18). Therefore, these three C-type lectin genes, which have analogous genomic structures (see below), form a tight cluster on human chromosome 19p13. We probed the PAC clones to search for additional related

FIGURE 1. Alignment of the predicted proteins, DC-SIGN and DC-SIGNR. The predicted proteins show 77% identity at the amino acid level. Potential *N*-linked glycosylation sites (*NXT/S*) (17) and possible cytoplasmic functional motifs (*LL*, di-leucine motif; *YXXL*, potential internalization motif) (16, 23, 24) are annotated. The beginning of each of the following is marked as follows: *C*, cytoplasmic domain; *Tm*, transmembrane domain; *NR*, neck repeats (boxed); *CRD*, carbohydrate recognition domains (bold type). In the previously published partial DC-SIGNR cDNA sequence (AB015629) (4), intron 7 had not been spliced out of the 3′ untranslated region, and exons 2 and 6 were absent (sequences: *1*, DC-SIGN; *2*, DC-SIGNR).

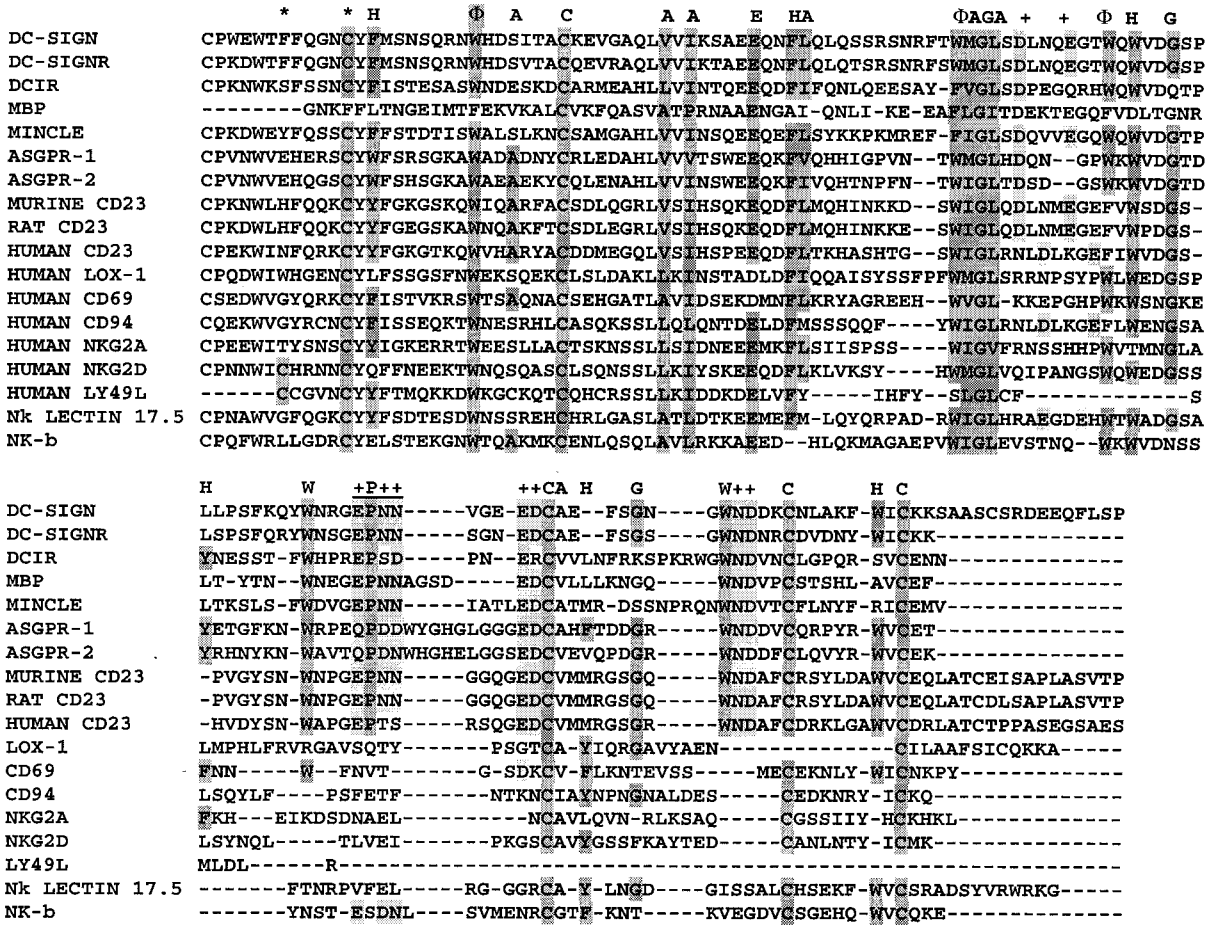


FIGURE 2. Aligment of carbohydrate recognition domains of DC-SIGN and DC-SIGNR with other C-type lectins. Residues conserved among C-type lectins are shown in dark gray. *H*, hydrophobic; *A*, aliphatic; *C*, cysteine; *G*, glycine; *E*, glutamic acid; *W*, tryptophan; *ϕ*, aromatic amino acid. Residues involved in calcium-dependent binding of carbohydrates (+) are shown in light gray. Those in the region marked +P++ determine the carbohydrate binding specificity. EDN mediates mannose/acetylglucosamine binding and QPD glucose/galactose specificity (31). DC-SIGN and DC-SIGNR possess all the residues required for calcium-dependent binding of mannose. Rat and mouse CD23 also contain these residues, although they are also able to bind protein (32). Human CD23 contains the majority of these motifs (18). NK cell lectins and proteins that bind *N*-acetylglucosamine and galactose via a conserved QPD motif, such as the asialoglycoprotein receptors (20), are shown for comparison. ∗, cysteine residues usually conserved in NK cell receptors (31). Accession numbers are as follows: human DC-SIGN, M98457; human DC-SIGNR, AF245219; human DCIR, CAB54001; MBP (human mannose binding protein precursor) (NP 000233); human mincle, BAA83755.1; ASGPR-1 (human asialoglycoprotein receptor-1), NP 00162; ASGPR-2 (human asialoglycoprotein receptor-2), CAA38997; murine CD23, P20693; rat CD23, S34198; human CD23, A10542; human Lox-1, CAB38175; human CD69, NP 001772; human CD94, BAA24450; human NKG2A, AAD03419; human NKG2D, CAA38652; human Ly49L, AAD44160; chicken hepatic lectin 17.5(I50146); chicken B-lectin, CAA18960.

genes using an exon 5 probe from DC-SIGN, which shows 93% identity to exon 5 of DC-SIGNR and <25% identity to CD23 at the nucleic acid level. Only two equimolar bands corresponding to DC-SIGN and DC-SIGNR were obtained on the PACs already identified (Fig. 3). Although we cannot rule out the presence of further closely related genes elsewhere in the genome, no additional EST sequences are currently identifiable, and high throughput genomic sequence data have revealed no evidence for this. It is therefore most likely that only two genes exist in the DC-SIGN family at 19p13.

Gene structure of DC-SIGN and DC-SIGNR

The complete genes for DC-SIGN and DC-SIGNR were cloned by PCR from genomic DNA derived from a B cell lymphoma line and sequenced. Coding regions were predicted by alignment to the sequences obtained from placental cDNA. Both genomic sequences showed 100% identity to the corresponding cDNAs over the coding regions. Exon/intron structures of the two genes are shown in Fig. 4. All intron-exon boundaries follow the ag/gt rule (Fig. 4). The genes have very similar structures, except that DC-SIGNR has a longer 3′ untranslated region, spanning two exons, compared with one in DC-SIGN. DC-SIGNR also contains an insert of approximately 1400 bp between exons 2 and 3, making intron 2 approximately 2000 bp, compared with 626 bp in DC-SIGN.

Expression pattern

Tissue specificity of expression was investigated by RT-PCR using gene-specific primers for *DC-SIGN* and *DC-SIGNR* (Fig. 5). In agreement with previous results (3), expression of *DC-SIGN* was restricted to endometrium, placenta, and stimulated KG1 cells (phenotypically similar to myeloid dendritic cells) (11). Although *DC-SIGNR* showed a lower level of expression, it was consistently detected in placenta with a very low level of expression in endometrium and stimulated KG1 cells. Cultured dendritic cells with a Langerhans cell-type phenotype (22) were negative for both molecules. We demonstrated a low level of expression of both DC-SIGN and DC-SIGNR by RT-PCR from monocyte-derived dendritic cells (data not shown) and subsequently cloned full-length cDNAs corresponding to both transcripts.

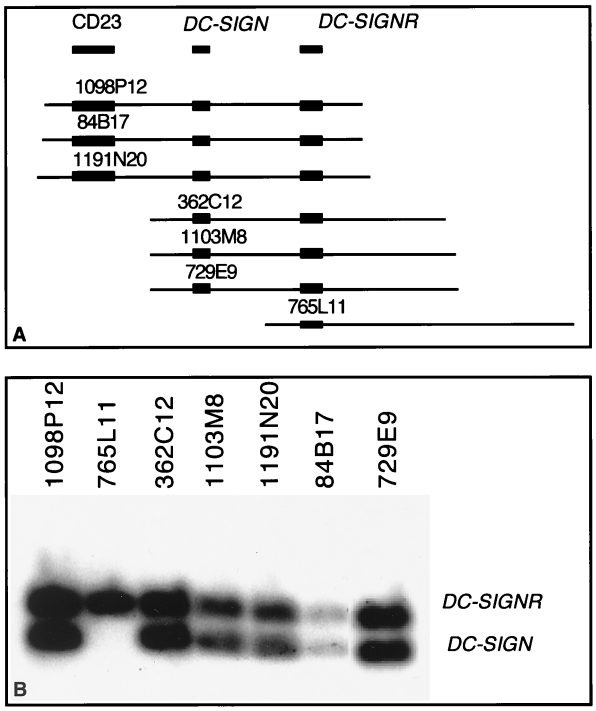
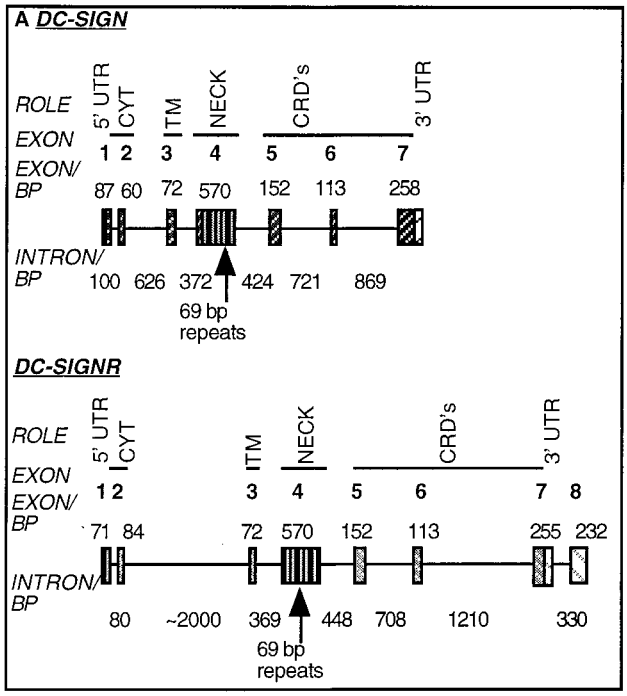


FIGURE 3. *A*, Alignment of PAC clones hybridizing with *DC-SIGN*. The RPCI 1–5 PAC libraries were screened with the full-length cDNA for *DC-SIGN*. Subsequent PCR confirmed that seven PACs contained *DC-SIGNR*, of which six contained *DC-SIGN* and three contained CD23 by PCR, consistent with the gene order shown. The smallest PACs containing all three genes are 105 kb. PAC sizes are as follows: 1098P12, 105 kb; 84B17, 105 kb; 1191N20, 110 kb; 362C12, 97 kb; 1103M8, 100 kb; 729E9, 108 kb; 765L11, 108 kb. *B*, Southern blot of PACs. A 300-bp probe was used to a conserved region using primers flanking exon 5 of the *DC-SIGN* gene. Digestion of all the *DC-SIGNR*-positive PACs was conducted with *Eco*RI and *Pst*I, which did not cut within the probe sequence. There was no evidence for additional closely related genes on these PACs.



B DC-SIGN

3' Intron Sequence (donor splice site)	Exon	5' Intron Sequence (acceptor splice site)
*gtgac*ATGAG	1	CCTGG**gt**gag
ttc**ag**AGGAG	2	AGCAG**gt**aga
ctc**ag**GGTGT	3	CCAAG**gt**cag
ccc**ag**TGTCC	4	AGTGG**gt**gag
aac**ag**AACGC	5	AGCAG**gt**aca
tcc**ag**AACTT	6	CCCAG**gt**aga
tgc**ag**CTTCA	7	

DC-SIGNR

3' Intron Sequence (donor splice site)	Exon	5' Intron Sequence (acceptor splice site)
*aaaac*ATGAG	1	CCTGG**gt**aag
ttc**ag**AAGAA	2	TACAG**gt**aag
ctc**ag**GGTGT	3	CCAAG**gt**cag
gtg**ag**TTGCC	4	AGTGG**gt**gag
aac**ag**AACGC	5	AGCAG**gt**aca
tcc**ag**AACTT	6	CCCAG**gt**aga
tgc**ag**CTTCC	7	CCACT**gt**aa
*tcc**ag**CTACA*	*8*	

FIGURE 4. *A*, *DC-SIGN* and *DC-SIGNR* gene structures. These were assembled by comparison of the full genomic sequence of *DC-SIGN* and partial sequence of *DC-SIGNR*. The two genes have very similar gene structures, except for the presence of an additional 3′ untranslated exon of *DC-SIGNR*. This is present in cDNA. Sequence data across intron 2 of *DC-SIGNR* are incomplete. Sizes are shown in nucleotides. *CYT*, cytoplasmic domain; *TM*, transmembrane domain. Note that in the previously published partial *DC-SIGNR* cDNA sequence AB015629 (4), intron 7 had not been spliced out of the 3′ untranslated region. *B*, Intron-exon boundaries of *DC-SIGN* and *DC-SIGNR*. All intron-exon boundaries follow the ag/gt rule. Initiating ATGs are underlined. 5′ and 3′ untranslated regions are shown in italics. Exons are written in capital letters. Introns are written in lower case letters. AG/GT splice donor and acceptor sites are shown in bold type. *DC-SIGN* has a short 3′ untranslated region, encoded by only one exon and consisting of 59 bases, while the *DC-SIGNR*s 289-bp 3′ untranslated region is encoded by two exons.

Discussion

This study describes the comparative sequences, genomic organization, mapping to chromosome 19p13, and expression analysis of two related C-type lectin genes, *DC-SIGN* and a novel, closely related gene, *DC-SIGNR*. These two genes and CD23, the low affinity receptor for IgE, are all encoded within a 105-kb region of 19p13. The *DC-SIGN*, *DC-SIGNR*, and *CD23* genes all possess CRDs encoded by three separate exons. The close linkage and similar gene structures suggest that these three genes may have arisen via duplication of an ancestral gene.

The cytoplasmic tails of both DC-SIGN and DC-SIGNR contain di-leucine motifs, which may mediate internalization, thus indicating that these molecules may act as carbohydrate receptors (Fig. 1) (23). However, work by Geijtenbeek et al. suggests that endocytosis via DCSIGN is extremely inefficient in dendritic cells (2). A YXXL motif in the cytoplasmic tail of DC-SIGN (Fig. 1) may provide a further internalization motif (16) or, alternatively, a site for tyrosine phosphorylation (24).

CD23 has a neck consisting of three leucine-rich repeats in man and two in rat and mouse. This forms an α-helical coiled coil structure, which mediates trimerization (25, 26). Each of the repeat units is 21 aa long and is encoded by a separate exon. This contrasts with *DC-SIGN* and *DC-SIGNR*, which each have 7 repeats of a 23-aa sequence, encoded by a single exon (Fig. 4). There is very high sequence identity between the repeat units, within each protein, and between *DC-SIGN* and *DC-SIGNR* (Fig. 1). By analogy to other lectin receptors, such as the asialoglycoprotein receptors and CD23 (26–29), we suggest that this domain could mediate oligomerization, forming an α-helical coiled coil. Oligomerization may serve to increase the avidity of ligand binding. The human

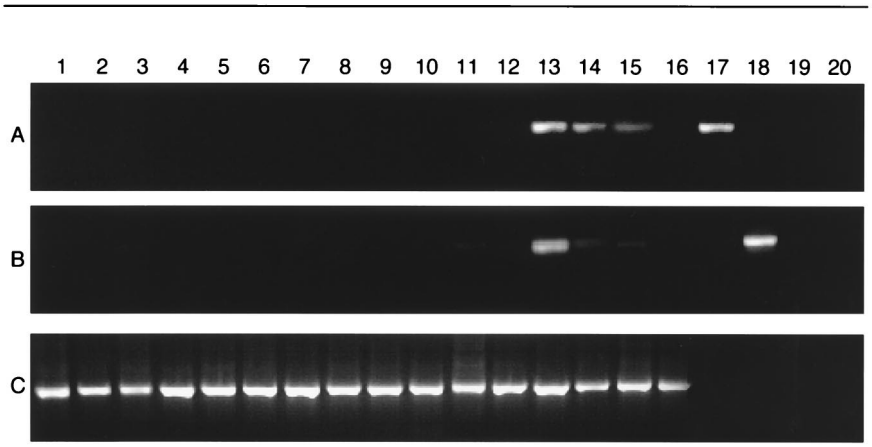


FIGURE 5. *DC-SIGN* and *DC-SIGNR* gene-specific RT-PCR. Expression of *DC-SIGN* was restricted to placenta, endometrium, and stimulated KG1 cells (which are phenotypically similar to myeloid dendritic cells) (11) in agreement with previous results (3). Although *DC-SIGNR* expression was lower, it was consistently detected in placenta with very low expression in the endometrium and stimulated KG1 cells. Cultured dendritic cells with a Langerhans cell-type phenotype (22) were negative for both molecules. *A*, *DC-SIGN*; *B*, *DC-SIGNR*; *C*, GAPDH. *Lane 1*, U937 (monocyte cell line); *lane 2*, Jurkat (T cell line); *lane 3*, NKL (NK cell line); *lane 4*, YT (NK/T cell line); *lane 5*, Ind (B cell line); *lane 6*, peripheral blood leukocytes; *lane 7*, T47D (breast carcinoma line); *lane 8*, HT29 (colon carcinoma line); *lane 9*, MRC5 (fibroblast line); *lane 10*, C33a (E6-transformed keratinocyte line); *lane 11*, 293T (embryonic kidney cell line); *lane 12*, HVMED-E6 (E6 transformed vascular smooth muscle cell line); *lane 13*, term placenta; *lane 14*, endometrium; *lane 15*, PMA/ionomycin-stimulated KG1 cells; *lane 16*, Langerhans-type dendritic cells; *lane 17*, *DC-SIGN* cloned cDNA; *lane 18*, *DC-SIGNR* cloned cDNA; *lane 19*; water; *lane 20*, genomic DNA.

asialoglycoprotein receptors, H1 and H2, have similar coiled coil neck structures and have been shown to form noncovalently associated heterotetramers with a stoichiometry of 2:2 (28). Like *DC-SIGN* and *DC-SIGNR*, the H1 and H2 genes are linked, but are found on chromosome 17p (30).

The sequences of the CRDs of *DC-SIGN* and *DC-SIGNR* show greatest identity to the human asialoglycoprotein receptors (41 and 34% at the amino acid level, respectively) and rat CD23 (both 33% at the amino acid level; Fig. 2). Consistent with previous work (1–3), *DC-SIGN*, shows features of a mannose binding lectin, as opposed to the features of a protein-binding NK cell lectin (Fig. 2) (31). *DC-SIGNR*, shows 77% identity to *DC-SIGN* at the amino acid level and also possesses all the residues shown to be required for the binding of mannose (Fig. 2) (31). The high level of homology between *DC-SIGN* and *DC-SIGNR* and their concomitant expression in placenta, endometrium, and a subset of dendritic cells suggest that DC-SIGNR may function in a similar manner to DC-SIGN, binding HIV gp120, ICAM-3 and perhaps other mannosylated proteins. Although it has been shown that the binding of DC-SIGN to ICAM3 and gp120 can be inhibited by mannan (1, 3) and may therefore not involve direct protein-protein interactions, the presence of the residues required for mannose binding must be interpreted cautiously. These residues are largely conserved in human CD23 and are completely conserved in murine and rat CD23 (Fig. 2), although the primary ligand of these molecules is thought to be IgE (18). Data suggest that mannose can partially inhibit the binding of human CD23 to certain of its ligands (32, 33). Therefore, DC-SIGN and DC-SIGNR may also bind a protein ligand, and experiments are underway to investigate this possibility.

The existence of a *DC-SIGN*-related gene (*DC-SIGNR*) with a similar pattern of expression is intriguing. Besides the formation of homo-oligomers, it is possible that hetero-oligomers of the two polypeptides may form, as described for the human asialoglycoprotein receptors (28). Given the heterogeneity of dendritic cell phenotypes (34), further investigation of the differential expression patterns of the two genes across these phenotypes and at different anatomical sites is warranted. A further question is posed by the high level of expression in placenta, which may not be accounted for entirely by the presence of cells with a dendritic cell phenotype. The expression of DC-SIGN or DC-SIGNR on placenta may provide the key to explaining the mechanism of vertical transmission of HIV and may therefore give valuable insights into its prevention.

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