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# Hodgkin's Lymphoma Cell Lines Express a Fusion Protein Encoded by Intergenically Spliced mRNA for the Multilectin Receptor DEC-205 (CD205) and a Novel C-type Lectin Receptor DCL-1*

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**Classic Hodgkin's lymphoma (HL) tissue contains a small population of morphologically distinct malignant cells called Hodgkin and Reed-Sternberg (HRS) cells, associated with the development of HL. Using 3′-rapid amplification of cDNA ends (RACE) we identified an alternative mRNA for the DEC-205 multilectin receptor in the HRS cell line L428. Sequence analysis revealed that the mRNA encodes a fusion protein between DEC-205 and a novel C-type lectin DCL-1. Although the 7.5-kb DEC-205 and 4.2-kb DCL-1 mRNA were expressed independently in myeloid and B lymphoid cell lines, the DEC-205/DCL-1 fusion mRNA (9.5 kb) predominated in the HRS cell lines (L428, KM-H2, and HDLM-2). The DEC-205 and DCL-1 genes comprising 35 and 6 exons, respectively, are juxtaposed on chromosome band 2q24 and separated by only 5.4 kb. We determined the DCL-1 transcription initiation site within the intervening sequence by 5′-RACE, confirming that DCL-1 is an independent gene. Two DEC-205/DCL-1 fusion mRNA variants may result from cotranscription of DEC-205 and DCL-1, followed by splicing DEC-205 exon 35 or 34–35 along with DCL-1 exon 1. The resulting reading frames encode the DEC-205 ectodomain plus the DCL-1 ectodomain, the transmembrane, and the cytoplasmic domain. Using DCL-1 cytoplasmic domain-specific polyclonal and DEC-205 monoclonal antibodies for immunoprecipitation/Western blot analysis, we showed that the fusion mRNA is translated into a DEC-205/DCL-1 fusion protein, expressed in the HRS cell lines. These results imply an unusual transcriptional control mechanism in HRS cells, which cotranscribe an mRNA containing DEC-205 and DCL-1 prior to generating the intergenically spliced mRNA to produce a DEC-205/DCL-1 fusion protein.**

Classic Hodgkin's lymphoma (HL)[1] is a common malignant lymphoma characterized by the presence of a small population (<1%) of putative malignant cells, the morphologically distinct Hodgkin and Reed-Sternberg (HRS) cells. Recent advances in cell isolation techniques and molecular biology has identified Ig gene rearrangements within the majority of individual HRS cells, suggesting their B cell origin (1). These are surrounded by a large population of apparently non-malignant lymphocytes and histiocytes, whose proliferation is likely to be mediated by the wide range of cytokines and chemokines released by the HRS cells (reviewed in Refs. 2 and 3).

HRS cells have many characteristics in common with antigen-presenting cells (APCs) such as activated B cells and dendritic cells (DCs) (4). Indeed, the HRS cell lines (L428, HDLM-2, and/or KM-H2) express cell surface molecules required for costimulation/proliferation of T cells (major histocompatibility complex class II, CD40, CD80, and CD86) (5–7), cell adhesion molecules involved in DC-T cell interactions (LFA-1, CD11c, and ICAM-1–3) (8, 9), and the DC-associated molecules (CD83 and fascin) (6, 10). They also produce inflammatory cytokines (*e.g.* tumor necrosis factor-$\alpha$ and lymphotoxin) (11), non-inflammatory cytokines (*e.g.* granulocyte macrophage-colony stimulating factor and interleukins 5 and 13) (12, 13), and chemokines (*e.g.* TARC) (14), which are associated with APCs. L428 cells have been used successfully in our laboratory to produce monoclonal antibodies (mAb) against DC differentiation antigens such as CMRF-44 (15) and CMRF-56 (16) and to clone the DC-associated molecules such as DEC-205 type I transmembrane multilectin receptor (17) and the adenosylhomocysteine hydrolase-like molecule DCAL/AHCYL-1 (18).

We have investigated cell surface molecules on HRS cell lines with a view to identifying novel molecules related to APC function. These molecules might also be candidate targets for antibody-based HL immunotherapy. Indeed, CD20, CD25, and CD30 reagents (markers for B cells and activated lymphocytes) have been investigated in this regard (19–21), but molecules more restricted to HRS cells might be preferred as targets for more specific therapeutics.

During the cloning of DEC-205 from the L428 cell line by 3′-rapid amplification of cDNA ends (RACE) (17), we discovered an alternatively spliced novel DEC-205 mRNA. This mRNA encodes the intact DEC-205 ectodomain but included unique sequences encoding for an additional carbohydrate recognition domain (CRD) and a transmembrane (TM) and a cytoplasmic (CP) domain derived from a newly identified type I transmembrane C-type lectin DCL-1. A partial cDNA sequence

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*The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY184222, AY314006, and AY314007.*

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[1] The abbreviations used are: HL, Hodgkin's lymphoma; HRS, Hodgkin and Reed-Sternberg; APC, antigen-presenting cell; DC, dendritic cell; RACE, rapid amplification of cDNA ends; RT, reverse transcriptase; CRD, carbohydrate recognition domain; TM, transmembrane domain; CP, cytoplasmic domain; HRP, horseradish peroxidase; mAb, monoclonal antibody; MMR, macrophage mannose receptor; $PLA_2R$, phospholipase $A_2$ receptor; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; SP, signal peptide.

TABLE I
*The DNA sequences of oligonucleotides primers used in this study*

| Primer | Sequence (5′→3′) | Accession number | Position (orientation) |
|---|---|---|---|
| 061 | CATCTGGGCCTTTCCATTGCT | AY314007 | 286–306 (reverse) |
| 062 | GACCATGGAGCGGACATGATA | AY314007 | 216–236 (forward) |
| 063 | GGCTCTACCATCTGGGTTTGT | AY314007 | 1811–1831 (forward) |
| 078 | GAAATGGTTGACTACAAAGAAGA | AF011333 | 4200–4222 (forward) |
| 085 | ACCAAATCAGTCCGCCCATGAGAA | AF011333 | 5095–5118 (reverse) |
| 086 | ATCATGTCCGCTCCATGGTCAGTA | AY314007 | 212–235 (reverse) |
| 088 | TATTCAGAAGTTAAAAGCAGA | AF011333 | 3327–3347 (forward) |
| 090 | CCAAAAGGCCGTACTCCAAAA | AF011333 | 2430–2450 (forward) |
| 092 | GGAGGAAAACTGAATGACGCA | AF011333 | 1518–1538 (forward) |
| 094 | GAAAACGGTTGTGAAGATAAT | AF011333 | 690–710 (forward) |
| 199 | GCTCCATGGTCAGTACACTGA | AY314007 | 206–226 (reverse) |

(KIAA0022) of DCL-1 was identified by random sequencing of a KG-1 cDNA library (22). Here, we describe the characterization of the DEC-205/DCL-1 fusion mRNA and protein. Its apparently selective expression in HRS cells may make it a useful target for both antibody- and T cell-mediated immunotherapy.

## EXPERIMENTAL PROCEDURES

*Cell Lines*—The human hematopoietic cell lines, HEL, KG-1, K562, THP-1, U937, Mann, Daudi, Raji, WT49, Mann, Molt-4, Jurkat, HL-60, and HSB-2 were obtained from the American Type Culture Collection (Rockville, MD). L428 cells were provided by V. Diehl (Klinik fur Innere Medizin, Cologne, Germany) (23). HDLM-2 (24) and KM-H2 cells (25) were obtained from the German Collection of Microorganism and Cell Culture (Braunschweig, Germany). Mono Mac 6 cells (26) were provided by E. M. Schneider (Dusseldorf, Germany). All cell lines were maintained in RPMI 1640 (Invitrogen, Melbourne, Victoria, Australia), 10% (v/v) fetal calf serum (Invitrogen), 100 units/ml penicillin, and 100 $\mu$g/ml streptomycin, except for HDLM-2 cells, which were maintained in 20% (v/v) fetal calf serum. These cells were subjected to RNA preparation using TRIzol (Invitrogen) for RT-PCR and Northern blot analysis.

*Antibodies and Other Reagents*—The mAb MMRI-7 against human DEC-205 was produced in our laboratory (27). MMRI-7 binds to an epitope within DEC-205 CRDs 1 and 2. The other anti-human DEC-205 mAb, M335, was provided by R. J. Armitage (Immunex, Seattle, WA) through the 7th International Workshop on Human Leukocyte Differentiation Antigens. M335 binds to an epitope within DEC-205 cysteine-rich domain (27).

Goat anti-mouse IgG was purchased from Dako (Botany, New South Wales, NSW, Australia). Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG-Fc-specific and protein A-conjugated agarose beads were from Sigma (Castle Hill, NSW, Australia). HRP-conjugated sheep anti-rabbit IgG was from Silenus (Melbourne, Victoria, Australia). ELISA plates (Maxsorb) were from Nalge Nunc International (Rochester, NY). Prestained protein standards (Benchmark Prestained Protein Ladder) and DNA ladder (1-kb ladder) were from Invitrogen. Molecular biological enzymes (*e.g.* restriction enzymes, polymerases, and ligase) were obtained from Invitrogen, Promega (Sydney, NSW, Australia) or Roche Applied Science (Castle Hill, NSW, Australia). Unless specified, general chemicals were obtained from Sigma or BDH (Poole, England).

Rabbit polyclonal peptide antisera against the DEC-205 CP domain and the DCL-1 CP were produced by immunizing New Zealand White rabbits with diphtheria toxoid-conjugated synthetic peptide CEDEIMLPSFHD and CGEENEYPYQFD (Minotopes, Clayton, Victoria, Australia), respectively, using a conventional schedule with Freund adjuvant at the Herston Medical Research Institute (Herston, Queensland, Australia). To assess the titer of the antibodies against CP peptides, an ELISA plate was coated with streptavidin (Sigma) and biotinylated peptides for DEC-205 CP (biotin-SGSGEDEIMLPSFHD) and DCL-1 CP (biotin-SGSGEENEYPYQFD) captured. The plate was blocked with 1% (w/v) sodium caseinate (Sigma) in PBS and 0.1% (w/v) Tween 20 (PBS/Tween) and incubated with serially diluted antisera. After washing the plate with PBS/Tween, bound antibody was detected with HRP-sheep anti-rabbit IgG and *o*-phenylenediamine hydrochloride and quantitated with 492 nm using an ELISA reader. There was no cross-reactivity detected between these two rabbit CP antibodies at the dilutions used in the experiments described (data not shown).

*3′-Rapid Amplification of cDNA Ends*—The 3′-end of DEC-205 mRNA was obtained by 3′-RACE, which was performed as described previously (17). Briefly, L428 mRNA was reverse-transcribed with an oligo(dT) adaptor primer. The obtained L428 cDNA pool was subjected to PCR using a DEC-205-specific forward primer and an adaptor primer and cloned into pBluescript SKII (Stratagene, La Jolla, CA). The clones were analyzed by restriction enzyme mapping and sequencing using a BigDye Terminator kit on an ABI Prism 377 automated sequencer (PE Applied Biosystems, Scoresby, Victoria, Australia) by Australian Genome Research Facility (University of Queensland, St. Lucia, Queensland, Australia).

*RT-PCR Analysis*—PCR was performed on the L428 cDNA pool using DEC-205-specific forward primers (078, 088, 090, 092, and 094, nested within various parts of the DEC-205 ectodomain) in combination with either DEC-205-specific reverse primer (085, nested within DEC-205 CP) or DCL-1-specific reverse primer (086, nested within DCL-1 ectodomain) with an Expand Long Template PCR system (Roche Applied Science) (Table I). The PCR reactions were fractionated in 0.8% (w/v) agarose in Tris acetate buffer (40 mM Tris acetate, 1 mM EDTA, pH 7.6) and visualized with ethidium bromide. The PCR products obtained by the primer combination 078/085 and 078/086 were cloned into pGEM-T Easy vector (Promega) and sequenced.

*Northern Blot Analysis*—Approximately 10 $\mu$g of total RNA from cultured cell lines was fractionated in formaldehyde-denatured 1% (w/v) agarose gel and transferred to a Hybond $N^+$ cationic nylon membrane (Amersham Biosciences, Sydney, NSW, Australia). The 864-bp DEC-205 cDNA probe nested within DEC-205 CRD1 and -2 was PCR-amplified using primers 094 and 095 on the DEC-205 cDNA clone pCRD1/2-Ig (27) and *Taq* polymerase (Roche Applied Science). The 1617-bp DCL-1 cDNA probe was PCR-amplified using DCL-1-specific primers 062 and 063 on the pBS30-1 (Fig. 1). These probes were purified using a QIAquick PCR purification kit (Qiagen, Clifton Hill, Victoria, Australia) and labeled with [$\alpha$-$^{32}$P]dATP (Amersham Biosciences) using a Strip-EZ DNA StipAble DNA probe Synthesis and Removal kit (Ambion, Austin, TX). The membrane was hybridized sequentially with these probes and exposed to a Kodak BioMax MS x-ray film at −70 °C using an intensifying screen (Amersham Biosciences). The final wash was 0.1 × SSC (1 × SSC is 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) and 0.5% (w/v) SDS at 68 °C. After each probing, the membrane was chemically stripped according to the manufacture's instructions and used for hybridization with the other probes.

*5′-RACE*—RNA ligase-mediated 5′-RACE was performed using a FirstChoice RLM-RACE kit (Ambion). Briefly, total RNA from HL-60 was treated sequentially with calf intestinal alkaline phosphatase and tobacco acid pyrophosphatase to select and to remove the cap structure of full-length mRNA. The RNA adaptor was ligated to the RNA using T4 RNA ligase, and the RNA was subjected to cDNA synthesis with random decamer or DCL-1-specific primer 061 and Thermoscript reverse transcriptase (Invitrogen). The cDNA was subjected to two rounds of PCR using DCL-1-specific primers 086 and 099 in combination with the 5′-RACE outer primer and inner primer (provided by the kit), respectively. The PCR product was cloned into pGEM-T Easy vector and sequenced.

*Preparation of Cell Lysate*—Approximately $10^7$ cells were lysed with 1 ml of 0.15 M NaCl, 25 mM Tris-HCl, pH 7.4, 1% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, and a mixture of protease inhibitors (Complete, EDTA-free, Roche Applied Science) and incubated on ice for 10 min with occasional vortexing. After centrifugation at 12,000 × *g* for 20 min at 4 °C, the supernatant was collected and used directly for immunoprecipitation/Western blotting or sandwich ELISA analysis described below.

*Immunoprecipitation/Western Blot Analysis*—The cell extract was

precleared with a non-immune rabbit serum and protein A-Sepharose (Sigma) for 1 h at 4 °C and subjected to immunoprecipitation using the rabbit peptide antisera against DEC-205 CP or DCL-1 CP with protein A-Sepharose overnight at 4 °C. The beads were washed with a wash buffer (0.15 M NaCl, 25 mM Tris-HCl, pH 7.5, 0.2% (v/v) Triton X-100, and 0.5% (w/v) sodium deoxycholate), and eluted with SDS-PAGE sample buffer (2% (w/v) SDS, 62.5 mM Tris-HCl, pH 6.8, 0.01% (w/v) bromphenol blue, and 10% (v/v) glycerol) by heating at 95 °C for 5 min. The samples were subjected to Laemmli discontinuous SDS-PAGE with 10% (v/v) polyacrylamide separating gel (28) in the non-reducing condition and transferred to a polyvinylidene fluoride membrane (PVDF-Plus, Osmonics, Westborough, MA). The membrane was blocked with 5% (w/v) nonfat dry milk in PBS/Tween (BLOTTO), incubated with a mixture of DEC-205 mAbs (MMRI-7 and M335, 5 $\mu$g/ml each) overnight at 4 °C, and washed with PBS/Tween. The membrane was incubated with HRP-anti-goat mouse IgG, and the bound enzyme was detected with enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL) on a Kodak X-Omat XB-1 x-ray film.

*Sandwich ELISA*—An ELISA plate was coated with 10 $\mu$g/ml goat anti-mouse IgG in PBS, washed with PBS/Tween, and blocked with BLOTTO. To the plate a mixture of DEC-205 mAb (MMRI-7 and M335, 2 $\mu$g/ml each) was added and incubated for 1 h at room temperature. The plate was washed and incubated with the serially diluted cell extracts overnight at 4 °C. The plate was washed with PBS/Tween and incubated with either rabbit peptide antibodies against DEC-205 CP or DCL-1 CP (1:1000 dilution in PBS/Tween) or non-immune rabbit serum for 1 h at room temperature, and, after washing with PBS/Tween, the plate was incubated with HRP-conjugated goat anti-rabbit IgG in 5% mouse serum and PBS/Tween. The plate was developed with *o*-phenylenediamine dihydrochloride and quantitated at 492 nm.

## RESULTS

*Identification of the cDNA Clone Encoding DEC-205/DCL-1 Fusion*—To obtain the 3′-end of human DEC-205 mRNA, we performed 3′-RACE (17). This resulted in amplification of a PCR product of ~3 kb (data not shown). When we cloned the PCR product and analyzed several clones by restriction enzyme analysis, however, we realized that there were two distinct sequences within the PCR product. The clone pB30-3 contained the authentic DEC-205 sequence encoding the DEC-205 CRD 8–10, TM, and CP (17). The other clone pB30-1, however, encoded DEC-205 CRD 8–10 followed by a unique sequence distinct from the DEC-205 TM and CP sequence (Fig. 1*A*). The junction of the DEC-205 and unique sequence was located within the connecting region (spacer 11) between the DEC-205 CRD10 and TM. A BLAST search identified the unique sequence as a part of the cDNA, KIAA0022 derived from KG-1 cell cDNA library (22). Our further analysis showed that the KIAA0022 contained a partial cDNA encoding a novel type I transmembrane C-type lectin receptor, and we termed it DCL-1 (DEC-205-associated C-type Lectin-1). The complete DCL-1 coding region encodes a signal peptide (SP), one CRD, one TM, and one CP. The KIAA0022 cDNA was recently annotated to a C-type lectin molecule (GenBank™ accession number BAA03498), and its gene was mapped to chromosome band 2q24. More details of DCL-1 will be published elsewhere.[2]

The sequence analysis of the clone pB30-1 showed that fusion junction occurred within the codon G/GC ("/" indicates the junction) for Gly in the DEC-205 spacer 11, connected to the codon G/AC for Asp in the junction between the DCL-1 SP and CRD. The fusion junction was in-frame, connecting the DEC-205 CRD 10 to the DCL-1 CRD, TM, and CP, suggesting that the DEC-205/DCL-1 fusion mRNA is translated. Furthermore, analysis of the DEC-205 and DCL-1 genes indicated that for this fusion mRNA the junction is formed by splicing DEC-205 exon 35 and DCL-1 exon 1, resulting in the fusion of DEC-205 exon 34 to DCL-1 exon 2 (a variant fusion mRNA termed V34-2, Fig. 1*B*). An additional variant fusion mRNA termed V33-2 is described below.

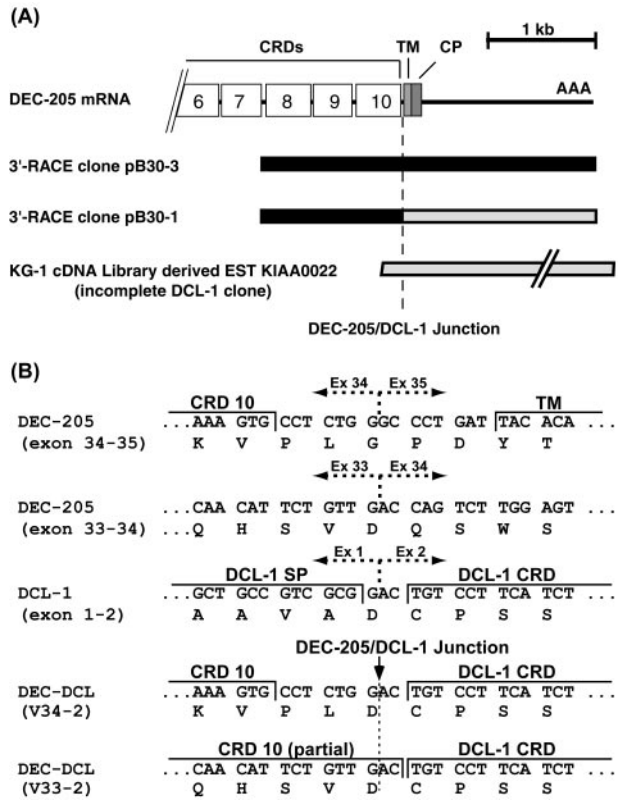


FIG. 1. **Identification of the cDNA clone encoding DEC-205/DCL-1 fusion.** *A*, a schematic presentation of DEC-205 mRNA (*top*, partial structure) and two representative clones (pB30-3 and pB30-1) isolated from the DEC-205 3′-RACE product. The *boxes* in the DEC-205 mRNA indicate domain structures, including carbohydrate recognition domains (CRDs), a transmembrane domain (TM), and a cytoplasmic domain (CP). *Wide black bars* indicate the DNA sequence for DEC-205 (17), and *wide shaded bars* indicate the DNA sequence for the novel C-type lectin DCL-1 (KIAA0022) (22). The *broken line* indicates the position of the junction between DEC-205 and DCL-1. *B*, the DNA and corresponding amino acid sequences adjacent to the junctions for two variants of DEC-205/DCL-1 fusion proteins. Top two sequences show DEC-205 sequences adjacent to the fusion junctions for DEC-205/DCL-1 fusion protein variants V34-2 and V33-2, respectively. The third sequence shows that the DCL-1 sequence adjacent to the fusion junction. The bottom two sequences show DEC-205/DCL-1 fusion variants V34-2 and V33-2 produced by DEC-205 exon 34 fused to DCL-1 exon 2 and DEC-205 exon 33 fused to DCL-1 exon 2, respectively. An *arrow* indicates the DEC-205/DCL-1 junction, apparent after gene analysis was performed to assign the exon-intron junction of DEC-205 and DCL-1 gene. *SP*, signal peptide.

*The DEC-205/DCL-1 Fusion mRNA Appears to Encode the Entire DEC-205 Ectodomain*—We examined the L428 cDNA pool containing the DEC-205/DCL-1 junction by RT-PCR to examine whether it included the entire DEC-205 ectodomain (Fig. 2). The combination of the DEC-205 CP-specific reverse primer 085 with DEC-205-specific forward primers, nested to various parts of DEC-205 ectodomain, yielded major PCR products of the sizes predicted in accordance with the primer combinations used. We also detected slightly smaller (by ~200 bp) minor PCR products, which were most apparent in the primer combinations of 078/085 and 088/085. When the DCL-1-specific reverse primer 086 was used in combination with the same DEC-205-specific forward primers, we detected doublet bands (~200 bp apart), the larger band of which was the predicted size. Sequence analysis indicated that the smaller RT-PCR fragments from DEC-205 itself or the DEC-205/DCL-1 fusion mRNA were amplified from alternatively spliced RNA, lacking

[2] S. Khan, K. J. McDonald, B. P. O'Neill, N. Gonzalez, B. J. Cooper, D. N. J. Hart, and M. Kato, manuscript in preparation.

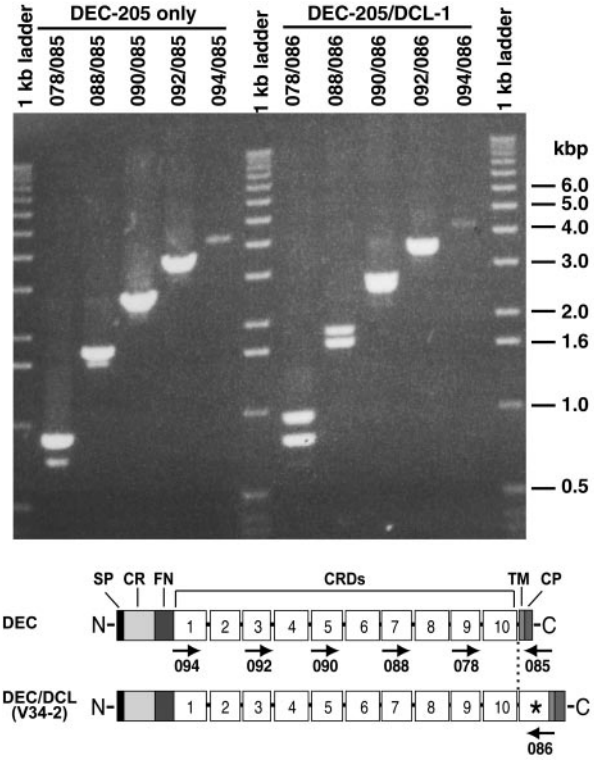


FIG. 2. **The DEC-205/DCL-1 fusion mRNA encodes the entire DEC-205 ectodomain.** The L428 cDNA was subjected to RT-PCR using either a DEC-205-specific reverse primer (085) or a DCL-1-specific reverse primer (086) in combination with various DEC-205-specific forward primers (078, 088, 090, 092, and 094) and fractionated with 0.8% (w/v) agarose gel electrophoresis. The positions of these gene-specific primers are indicated as *arrows* in the schematic diagram (*bottom*). The doublets obtained with several sets of primer combinations correspond to alternatively spliced DEC-205 mRNA (see text). *SP*, signal peptide; *CR*, cysteine-rich domain; *FN*, fibronectin type II domain; *CRD*, carbohydrate recognition domain; *TM*, transmembrane domain; *CP*, cytoplasmic domain.

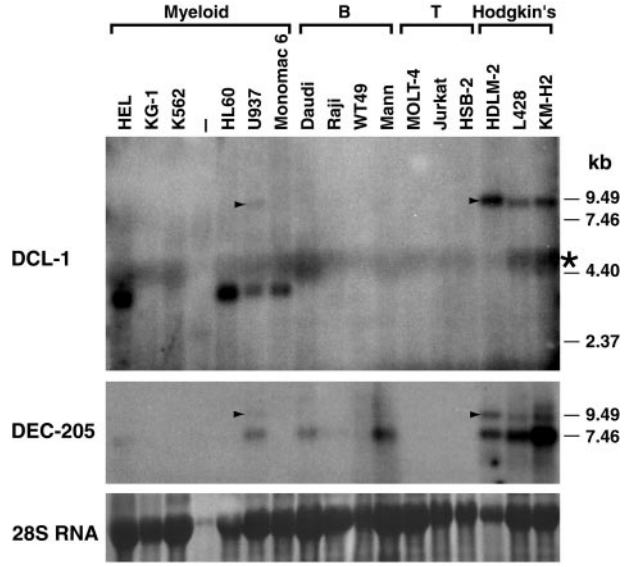


FIG. 3. **The DEC-205/DCL-1 fusion mRNA is predominantly expressed by HRS cell lines.** Total RNA from hematopoietic cell lines was subjected to Northern blot analysis, probed sequentially with the DCL-1 (*top panel*) and DEC-205 (*middle panel*). The *bottom panel* shows methylene blue staining of 28 S ribosomal RNA. The *arrowheads* indicate the positions of 9.5-kb DEC-205/DCL-1 fusion mRNA detected by both DCL-1 (*top panel*) and DEC-205 probes (*middle panel*). The *asterisks* indicate the positions of nonspecific signals caused by the nonspecific binding of DCL-1 probe to 28 S RNA.

DEC-205 exon 34 (168 bp, described below). Thus, L428 cells express at least two variants of the DEC-205/DCL-1 fusion mRNAs, one with DEC-205 exon 34 fused to DCL-1 exon 2 (a variant termed V34-2) and one with DEC-205 exon 33 fused to DCL-1 exon 2 (a variant termed V33-2) (Fig. 2). Sequence analysis of the fusion junction of V33-2 showed that the junction is in-frame, indicating that V33-2 DEC-205/DCL-1 fusion mRNA is also likely to be translated. The V34-2 encodes the entire DEC-205 ectodomain fused to DCL-1 CRD, TM, and CP. The V33-2 lacks approximately one-third of the C-terminal portion of DEC-205 CRD 10, and the rest of DEC-205 ectodomain is fused to DCL-1.

*The DEC-205/DCL-1 Fusion mRNA Is Predominantly Expressed by HRS Cell Lines*—To assess DEC-205/DCL-1 fusion mRNA expression, we performed Northern blot analysis in several hematopoietic cell lines (Fig. 3). The DCL-1-specific probe nested within the DCL-1 ectodomain detected a single 4.2-kb DCL-1 mRNA band in myeloid cell lines (HEL, HL60, U937, and Monomac 6), but no bands were detected in the B or T cell lines tested. We detected a single 9.5-kb DEC-205/DCL-1 mRNA band in HRS cell lines (HDLM-2, L428, and KM-H2), however, we did not detect the 4.2-kb DCL-1 mRNA band observed in the myeloid cell lines. The U937 cells appear to express a small amount of the 9.5-kb DEC-205/DCL-1 mRNA in addition to the 4.2-kb DCL-1 mRNA band. When the DEC-205-specific probe nested within the cysteine-rich domain was used to hybridize the same blot after the DCL-1 probe was stripped, a 7.5-kb DEC-205 mRNA band was detected in myeloid cell lines (HEL and U937), B cell lines (Daudi and Mann), and all HRS cell lines. In addition, we detected a 9.5-kb DEC-205/DCL-1 mRNA band in all HRS cell lines and the U937 as described previously (17). Thus, it appears that the DEC-205/DCL-1 fusion mRNA predominates in HRS cell lines.

*The DEC-205 and DCL-1 Genes Are Juxtaposed in Chromosome Band 2q24*—We mapped the DEC-205 gene (*LY75*) previously to the chromosome band 2q24 (17). The *KIAA0022*/DCL-1 gene was previously located to chromosome 2 (22) and further mapped recently to the identical chromosomal band in the NCBI UniGene data base. Using the NCBI Genome BLAST, we identified the human genomic contig NT 005151 containing both DEC-205 and the DCL-1 gene. Our sequence analysis showed that DEC-205 and DCL-1 genes consist of 35 and 6 exons, respectively, and the DEC-205 gene is localized ~5.4 kb upstream of the DCL-1 gene (Fig. 4).

*The DCL-1 Gene Is Independently Expressed from the DEC-205 Gene*—It is possible that the proposed DCL-1 gene is a part of DEC-205 gene and that the DCL-1 mRNA is generated by alternative splicing of DEC-205 mRNA driven by DEC-205 promoter. If this were the case, the DCL-1 5′-untranslated region should contain at least some DEC-205 gene sequences. To assess this possibility, we performed RNA ligase-mediated 5′-RACE using HL-60 total RNA and determined the DCL-1 transcription initiation site (Fig. 5). This procedure is designed to amplify cDNA only from full-length, capped mRNA, and suitable to determine the transcription initiation site. Two rounds of DCL-1-specific PCR amplification of the DCL-1 cDNA yielded a ~250-bp single band regardless of primers (random decamers or DCL-1-specific primer 061) for reverse transcription (Fig. 5*A*). Sequencing of the 5′-RACE product indicated that DCL-1 transcription initiation site is mapped to 44 bp upstream of DCL-1 translation start codon (ATG, A at +1) located within the 5.4-kb intervening sequence between DEC-205 and DCL-1 gene. Thus, the DCL-1 gene is transcribed independently from DEC-205 gene.

Therefore, the DEC-205 and DCL-1 fusion mRNA variants appear to be generated by cotranscription of both DEC-205 and

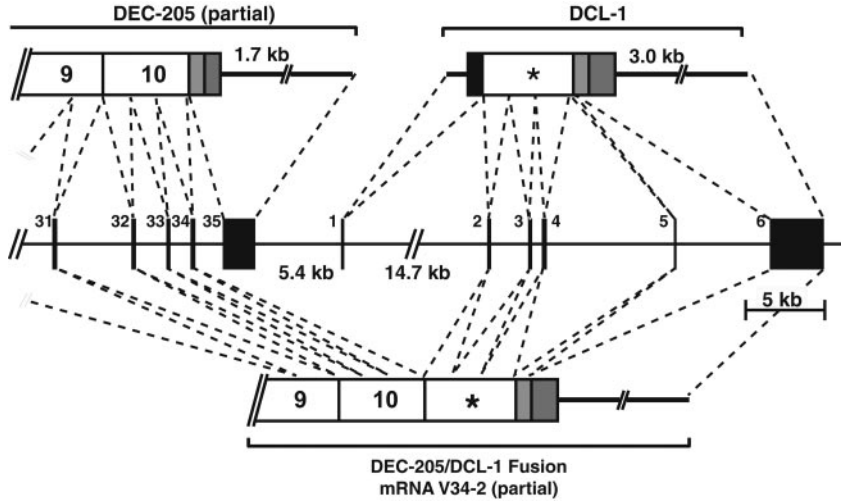


FIG. 4. **The DEC-205 and DCL-1 genes are juxtaposed in chromosome band 2q24.** A schematic drawing of DEC-205 (*partial*) and DCL-1 mRNA (*top*), DEC-205 (*partial*), and DCL-1 genes on chromosome 2q24 (*middle*) and DEC-205/DCL-1 fusion mRNA (V34-2, *bottom*). In the *top* and *bottom drawings*, *boxes* indicate domain structures (please see keys in Fig. 2). In the *middle panel*, *boxes* indicate exons. The drawing of DEC-205/DCL-1 fusion protein variant V33-2 is omitted for clarity.

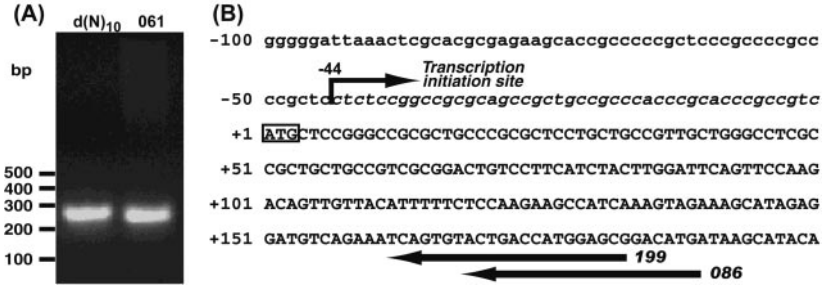


FIG. 5. **The DCL-1 gene is independently expressed from the DEC-205 gene.** Total RNA from HL-60 cells was subjected to RNA ligase-mediated 5′-RACE after being reverse-transcribed with random decamer ($d(N)_{10}$) or DCL-1-specific primer 061. *A*, the 5′-RACE product was fractionated with 2% agarose gel and stained with ethidium bromide. *B*, DNA sequence analysis of the DCL-1 transcription site. The 5′-RACE product was cloned and sequenced. The *lowercase letters* indicate the genomic DNA sequence upstream of DCL-1 translation start codon (ATG, *boxed*). A *kinked arrow* indicates the position for DCL-1 transcription initiation site. *Arrows* indicate the positions for DCL-1-specific primers (086 and 199) for two rounds of 5′-RACE amplification.

DCL-1 genes followed by intergenic splicing to remove the DEC-205 exon 35 alone or exon 34–35 along with DCL-1 exon 1, resulting in DEC-205 exon 34 fused to DCL-1 exon 2 (V34-2) or DEC-205 exon 33 fused to DCL-1 exon 2 (V33-2) (see Fig. 1). The DNA sequences of DEC-205/DCL-1 fusion mRNA variants and DCL-1 mRNA were submitted to the GenBank™ and assigned the accession number AY184222 (for V34-2), AY314006 (for V33-2), and AY314007 (for DCL-1), respectively.

*DEC-205/DCL-1 Fusion mRNA Is Translated to the Fusion Protein*—We sought to establish whether the DEC-205/DCL-1 fusion mRNA is translated into a fusion protein. We prepared cell lysates from three HRS cell lines (DEC-205 $mRNA^+$ and DEC-205/DCL-1 fusion $mRNA^+$), HEL (DEC-205 $mRNA^+$ and DEC-205/DCL-1 fusion $mRNA^-$), and Jurkat cell line (DEC-205 $mRNA^-$ and DEC-205/DCL-1 fusion $mRNA^-$) (see Fig. 3) and subjected them to immunoprecipitation with the DEC-205 CP or DCL-1 CP peptide antisera. The immunoprecipitated samples were further analyzed by Western blot with a mixture of DEC-205 mAbs (MMRI-7 and M335, both react to the N-terminal portion of DEC-205) to detect the DEC-205 and DEC-205/DCL-1 fusion proteins in non-reducing conditions (Fig. 6*A*). The DEC-205 CP antiserum precipitated a broad but single ~180-kDa DEC-205 protein band specifically from the three HRS cell lines (L428, HDLM-2, and KM-H2) and HEL cells. There was no detectable signal in Jurkat cells as expected. When the DCL-1 CP antiserum was used for the initial immunoprecipitation, we detected low levels of the ~180-kDa DEC-205/DCL-1 fusion protein band in the three HRS cell lines, but not in HEL or Jurkat cells, consistent with the expression of DEC-205/DCL-1 mRNA in these cell lines (see Fig. 3). The presence of this DEC-205/DCL-1 fusion protein band in these HRS cell extracts was not due to cross-reactivity of DCL-1 CP antiserum with DEC-205 CP, because (i) there was no cross-reactivity in the DCL-1 CP antiserum with DEC-205 CP peptide assessed by ELISA analysis (data not shown), (ii) 60 times longer exposure of HEL sample did not produce any band (Fig. 5*A*), and (iii) the DCL-1 CP antiserum detected the weakest signal in KM-H2 extracts, which contained most DEC-205 protein (Fig. 5*A* and described below). Reversibly, we were also able to detect DEC-205/DCL-1 fusion protein bands in HRS cell lines by immunoprecipitation with DEC-205 mAbs and protein G-conjugated beads and Western blot analysis with DCL-1 CP antiserum (data not shown).

To determine the relative abundance of the DEC-205/DCL-1 fusion protein to DEC-205, we developed a sandwich ELISA using the DEC-205 mAbs for capturing and the CP antisera for detection (Fig. 6*B*). The HRS cell lines express most DEC-205 protein (KM-H2 > L428 > HDLM-2), followed by HEL cells. We detected relatively small amounts of the DEC-205/DCL-1 fusion protein in L428 and HDLM-2 cells, ~30–50 times less than the amount of DEC-205. No fusion protein was detected in the KM-H2 cells, probably because the amount of KM-H2 derived fusion protein is below the detection limit. The negative control, Jurkat, did not show any signal. The relative abundance of both DEC-205 and DEC-205/DCL-1 fusion protein by the ELISA correlated with the immunoprecipitation/Western blot data (Fig. 6*A*).

## DISCUSSION

Cotranscription and intergenic splicing is a rare event in mammalian cells, and there are only a small number of reports describing the presence of fusion mRNA. These include *MDS1*

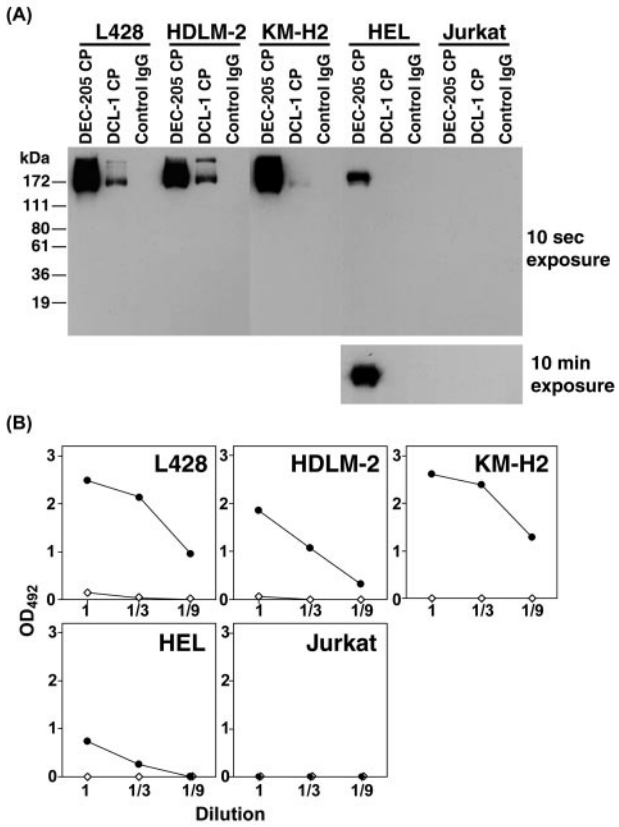


FIG. 6. **DEC-205/DCL-1 fusion mRNA is translated to the fusion protein.** *A*, the cell lysates from HRS cell lines (L428, HDLM-2, and KM-H2), HEL cells, and Jurkat cells were immunoprecipitated with anti-DEC-205 CP, anti-DCL-1 CP peptide antisera, or non-immune rabbit IgG, and the immune complexes were subjected to Western blot analysis using DEC-205 mAbs (MMRI-7 plus M335). The signals were detected by ECL on x-ray films. *B*, the cell lysates as above were applied to an ELISA plate coated with DEC-205 mAbs, and bound DEC-205 (*closed circles*) or DEC-205/DCL-1 fusion protein (*open diamonds*) was detected with anti-DEC-205 CP (for DEC-205) or anti-DCL-1 CP (for DCL-1), respectively. The signals were detected with *O*-phenylenediamine dihydrochloride at 492 nm.

*EVI1* (29), galactose-1-phosphate uridylyltransferase, and interleukin-11 receptor genes (30), *Prnd*/*Prnp* (31) and *P2Y1*/*SSF1* (32). None of these reports, however, examined whether the fusion mRNA is translated endogenously into cognate fusion protein. Here we describe another cotranscription and intergenic splicing of two juxtaposed genes, encoding two type I transmembrane C-type lectin receptors DEC-205 and DCL-1, respectively. Furthermore, we demonstrated for the first time to our knowledge that the fusion mRNA is translated endogenously into the DEC-205/DCL-1 fusion protein.

DEC-205 is a putative antigen uptake receptor expressed on dendritic cells (17, 33) and potent APCs, which initiate and direct immune responses (reviewed in Refs. 34–37). DEC-205 belongs to the macrophage mannose receptor family of endocytic receptors that include the prototype macrophage mannose receptor (MMR) (38, 39), phospholipase $A_2$ receptor ($PLA_2R$) (40, 41) and Endo180 (42, 43). The ectodomain of these receptors contains several domain structures, including a cysteine-rich domain, fibronectin type II domain, and multiple CRDs (10 for DEC-205 and 8 for others). The MMR, $PLA_2R$, and Endo180 exhibit C-type lectin activity (39, 40, 42), however, the DEC-205 ligands have yet to be identified. The cytoplasmic domain of these receptors contains either Tyr-based (in MMR, $PLA_2R$, and DEC-205) (39, 44, 45), di-aromatic amino acid-based (46), or di-hydrophobic amino acid-based (in Endo180) (47) motifs to facilitate their endocytosis to transport cognate ligand intracellularly. Although there are potential Ser and Thr phosphorylation sites within the CP of these lectins, no phosphorylation of these sites has been reported. In addition, the DEC-205 CP contains a cluster of acidic amino acids (EDE) that targets late endosomes, where loading of proteolytically processed antigenic peptides to major histocompatibility complex class II occurs (45). DCL-1 is a unique type I transmembrane C-type lectin in that DCL-1 ectodomain contains only one CRD, whereas other type I transmembrane C-type lectins contain more than one domain (*e.g.* selectins and MMR). DCL-1 CP contains several putative motifs, including a Tyr-based internalization, a cluster of acidic amino acids, and Ser and Tyr phosphorylation motifs, suggesting that DCL-1 CP mediates not only endocytosis and late endosome targeting but also signaling.[2]

The genes encoding DEC-205 (*LY75*) (17) and DCL-1 (*KIAA0022*) (22) are juxtaposed within chromosome band 2q24 and are separated by only ~5.4 kb (Fig. 4). These are independent genes, because DEC-205 and DCL-1 mRNA are each expressed independently in hematopoietic cell lines (Fig. 3). The 5′-RACE experiment mapped the DCL-1 transcription initiation site at 44 bp upstream of DCL-1 translation start codon (Fig. 5). Furthermore, our recent luciferase reporter assay studies showed that both 5′-proximal promoters of DEC-205 and DCL-1 have independent promoter activity (data not shown). The DEC-205 promoter may drive the cotranscription of the DEC-205 and DCL-1 genes to produce the 9.5-kb DEC-205/DCL-1 fusion mRNA. This would result from leaky termination of DEC-205 transcription, a mechanism suggested to explain the cotranscription of galactose-1-phosphate uridylyltransferase and interleukin-11 receptor genes (30). However, this seems unlikely, because the expression of DEC-205 mRNA did not correlate to that of DEC-205/DCL-1 fusion mRNA. Interestingly, all HRS cell lines tested expressed the 9.5-kb DEC-205/DCL-1 fusion mRNA, but not 4.2-kb DCL-1 mRNA, whereas other myeloid and B cell lines expressed 7.5-kb DEC-205 and/or 4.2-kb DCL-1 mRNA (Fig. 3), suggesting that expression of DEC-205/DCL-1 fusion mRNA is highly regulated. It is intriguing to speculate that HRS cell lines express certain transcription factors that may control cotranscription of DEC-205 and DCL-1 genes.

At mRNA levels, we identified two DEC-205/DCL-1 fusion mRNA variants (V34-2 and V33-2) different by the presence of the DEC-205 exon 34. The deletion of exon 34 appears to be the only alternative splicing that occurs naturally in DEC-205 gene transcription (Fig. 2). The fusion junctions in the V34-2 and V33-2 DEC-205/DCL-1 fusion mRNA are in-frame, suggesting both transcripts are translated.

What would be the functional difference between DEC-205 and DEC-205/DCL-1 fusion protein? Because the fusion protein contains DCL-1 CP, not DEC-205 CP, it is conceivable that DEC-205 ligand (currently unknown) to DEC-205/DCL-1 fusion protein would induce distinct signals from that binding to DEC-205. Further study of DEC-205 and DCL-1 is required to elucidate the function of these two C-type lectin receptors as well as DEC-205/DCL-1 fusion protein.

In this study, we used three independent HRS cell lines (L428, HDLM-2, and KM-H2) and showed that DEC-205/DCL-1 fusion mRNA is predominantly expressed in these HRS cell lines (Fig. 3) and that the mRNA is translated into a DEC-205/DCL-1 fusion protein (Fig. 5). We are currently investigating the presence of DEC-205/DCL-1 fusion mRNA in HL-affected lymph nodes *in situ*. If the expression of DEC-205/DCL-1 fusion protein is confirmed *in vivo* in HL, then the fusion protein may become relevant as a new target for antibody or T cell mediated immunotherapy for HL.

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# Hodgkin's Lymphoma Cell Lines Express a Fusion Protein Encoded by Intergenically Spliced mRNA for the Multilectin Receptor DEC-205 (CD205) and a Novel C-type Lectin Receptor DCL-1

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