Cloning of a Second Dendritic Cell-associated C-type Lectin (Dectin-2) and Its Alternatively Spliced Isoforms*

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Using a subtractive cDNA cloning strategy, we isolated previously five novel genes that were expressed abundantly by the murine dendritic cell (DC) line XS52, but not by the J774 macrophage line. One of these genes encoded a unique, DC-associated C-type lectin, termed “dectin-1.” Here we report the characterization of a second novel gene that was also expressed in a DC-specific manner. Clone 1B12 encoded a type II membrane-integrated polypeptide of 209 amino acids containing a single carbohydrate recognition domain motif in the COOH terminus. The expression pattern of this molecule, termed “dectin-2,” was almost indistinguishable from that for dectin-1; that is, both were expressed abundantly at mRNA and protein levels by the XS52 DC line, but not by non-DC lines, and both were detected in spleen and thymus, as well as in skin resident DC (i.e. Langerhans cells). Interestingly, reverse transcriptase-polymerase chain reaction and immunoblotting revealed multiple bands of dectin-2 transcripts and proteins suggesting molecular heterogeneity. In fact, we isolated additional cDNA clones encoding two distinct, truncated dectin-2 isoforms. Genomic analyses indicated that a full-length dectin-2 (α isoform) is encoded by 6 exons, whereas truncated isoforms (β and γ) are produced by alternative splicing. We propose that dectin-2 and its isoforms, together with dectin-1, represent a unique subfamily of DC-associated C-type lectins.

Dendritic cells (DC)† are far more potent than other antigen-presenting cells (e.g. macrophages and B cells) in their capacity to activate immunologically naive T cells, and DC are, indeed, responsible for initiating T cell-mediated immune responses to a variety of antigens (1, 2). Members of the DC family are distributed to virtually all the organs (except the brain), where they serve as tissue resident antigen-presenting cells, playing critical roles in presenting environmental, microbial, and tumor-associated antigens to the immune system.

Several years ago, we developed stable DC lines from the mouse epidermis (3). These lines, termed the XS series, maintain many important features of skin resident DC, i.e. Langerhans cells, and they have provided a useful tool for the application of modern technologies for studying DC biology (3–13). Most recently, we employed the subtractive cDNA cloning strategy to identify genes that were expressed preferentially by DC.² Briefly, we constructed a DC-specific cDNA library by subtracting cDNAs prepared from the XS52 DC line with excess amounts of mRNAs isolated from the J774 macrophage line. Following three rounds of screening of this library, we identified five novel genes that were expressed selectively by XS52 DC, but not by J774 macrophages. One of these genes encoded a type II membrane-associated polypeptide of 244 amino acids (αa) containing a single carbohydrate recognition domain (CRD) motif at the COOH-terminal end. This polypeptide was, therefore, designated as DC-associated C-type (Ca²⁺-dependent) lectin-1 or dectin-1. Dectin-1 mRNA was expressed in skin resident DC (from which the XS52 line was derived) as well as in spleen and thymus, the tissues known to contain relatively large numbers of DC.²

Although the physiological function of dectin-1 remains unknown at present, the identification of a novel, DC-associated lectin is of particular interest, because one of the characteristic features of DC is the expression of many C-type lectins (1, 2). For example, DC express DEC-205, a type I membrane-integrated glycoprotein that contains 10 distinct CRD motifs in the extracellular region (14). DC also express a macrophage mannose receptor (MMR), which is a type I membrane-associated glycoprotein with 8 CRD motifs (15). With respect to function, currently available data suggest that both DEC-205 and MMR mediate the uptake of glycosylated antigens by DC (1, 2, 15, 16). Unlike DEC-205 and MMR, which contain multiple CRD motifs in the NH₂-terminal ends, the second group of C-type lectins consists of the polypeptides that contain a single CRD in their COOH termini. Members of this group include: (a) hepatic lectins (HL) (or asialoglycoprotein receptors), (b) macrophage galactose/N-acetylgalactosamine-specific lectin (MGL), (c) CD23 (low affinity Fcε receptor), and (d) various receptors encoded in the natural killer gene complex (e.g. CD69, CD94, NKR-P1, Ly-49, and NKG2) (17, 18). Of these type II surface lectins, DC are known to express CD23 (19) and CD69 (20). More recently, Bates et al. (21) identified a novel, DC-associated type II surface lectin, termed DC immunoreceptor (DCIR). In summary, we now know that DC express both type I surface lectins (DEC-205 and MMR) and type II surface lectins (CD23, 69, DCIR, and dectin-1). In this study, we have identified new members of the DC-associated type II surface lectin family.

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The abbreviations used are: DC, dendritic cells; αa, amino acid(s); CRD, carbohydrate recognition domain; MMR, macrophage mannose receptor; HL, hepatic lectins; MGL, macrophage galactose/N-acetyl-galactosamine-specific lectin; DCIR, DC immunoreceptor; mAb, monoclonal antibody; RT, reverse transcriptase; PCR, polymerase chain reaction; nt, nucleotide(s); m, murine.

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**EXPERIMENTAL PROCEDURES**

**Animals**—Female BALB/c mice (6–10-week-old) and female Lou/c rats (6–20-week-old) were housed in the pathogen-free facility of the Animal Resource Center at the University of Texas Southwestern Medical Center. All the experiments were conducted according to the guidelines of the National Institutes of Health. To study tissue distributions of dectin-2 mRNA and protein, mice were sacrificed by overdose methohexital (inhalation). The lungs were inflated with PBS and frozen in liquid nitrogen.

**Cell Lines**—XS52 cells are long term DC line established from the epidermis of BALB/c mice (3). This line was maintained and expanded in complete RPMI 1640 supplemented with mouse recombinant granulocyte/macrophage-colony stimulating factor (1 ng/ml) and NS fibroblast culture supernatant (10% v/v) as a source of colony-stimulating factor-1 (3, 4). Other features of this DC line are described elsewhere (3–13). The J774 macrophage line derived from BALB/c mice was purchased from American Type Culture Collection (ATCC, Rockville, MD) and maintained in complete RPMI 1640 in the absence of added growth factors. We also used the Pam 212 keratinocyte line (22), NS fibroblast lines (3, 4, 23), 7–17 dendritic epidermal γδ T cell line (DETC) (24), rat macrophage line (ATCC, HDK-1 CD4 TH1 clone and D10 CD4 TH2 clone (kindly provided by Dr. Nancy Street, UT Southwestern Medical Center), 5C5 and 2G9 B cell hybridoma clones (provided by Dr. Mansour Mohamadzadeh, UT Southwestern Medical Center), and COS-1 line (ATCC).

**Epidermal Cell Isolation**—Epidermal cells were isolated from abdominal skin of BALB/c mice using two sequential trypsin treatment and then enriched for Langerhans cells by centrifugation over His-pelors (1,083, Sigma) as described previously (11, 25). Langerhans cell populations were separated from this preparation by anti-Ia mAb plus complement treatment as before (11).

**Isolation of Dectin-2 cDNA Clones**—A DC-specific cDNA library was constructed by subtracting the cDNA library prepared from the XS52 DC line with excess amounts of mRNAs isolated from the J774 macrophage line, and this library was screened by colony hybridization, slot blotting, and Northern blotting to identify the clones that were expressed selectively by the XS52 DC line (2). The original clone 1B12 was one of the 50 clones that were selected in the above manner. To isolate additional clones encoding dectin-2 isoforms, the DC-specific library was screened again by using clone 1B12 as a probe.

**Northern Blotting and RT-PCR Analyses**—Northern blotting was performed as described previously (26). Briefly, total RNAs (10 μg/lane) isolated from cell lines or mouse organs were fixed on a vertical agarose gel, transferred onto a nylon membrane, and hybridized with the 32P-labeled dectin-2 or GAPDH cDNA probe. RT-PCR was performed as before (11, 26) by using the following PCR primer sets: primer set 1, 5'-GGGGGTCATCTGTGGTGTT-3' and 5'-ATGGCCTCCGCTGTGCTCT-3'; primer set 2, 5'-ACCCCTGACCTTCTGAACAT-3'. Other PCR conditions were as described elsewhere (3–13).

**Preparation of Anti-Dectin-2 mAb**—Local/rats were immunized initially with recombinant His-dectin-2 proteins in complete Freund's adjuvant followed by bi-weekly injections of the same proteins in incomplete Freund's adjuvant. One week after the fifth immunization, spleens were harvested from these animals, and B cell hybridomas were prepared using the standard protocol (27). Culture supernatants were tested for the presence of antibodies reactive to His-dectin-2 proteins in immunoblotting. Positive clones were recloned by limiting dilution microculture. Immunoglobulin heavy and light chain genes were purified from these recombinant clones by affinity chromatography using anti-IgG-conjugated agarose.

**Immunoblotting**—XS52 DC were homogenized in 10 mM HEPES (pH 7.3) with 5–10 strokes with a 27-gauge needle on 1-ml syringe, centrifuged for 10 min at 1000 × g, and the resulting supernatant (‘crude lysate’) was fractionated into cytosolic and membrane fractions by centrifugation for 40 min at 100,000 × g. In some experiments, whole cell extracts were prepared from different cell lines in 0.3% Triton X-100 in phosphate-buffered saline followed by centrifugation for 10 min at 1000 × g. COS-1 cells were transfected using FuGene 6 (Roche Molecular Biochemicals) with pZeoSV2+ vector (Invitrogen, Carlsbad, CA) containing each of the three different dectin-2 cDNA clones; membrane fractions were prepared 72 h after transfection. These samples were separated by 10–20% or 4–20% SDS-polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA), and then blotted with anti-dectin-2 mAb R4C2 or control rat IgG. After an extensive wash, the membrane was blotted with horseradish peroxidase-conjugated anti-rat IgG (Zymed Laboratories Inc., CA) and then developed with ECL system (Amersham Pharmacia Biotech).

**Genomic Screening and Subcloning**—A mouse genomic library (strain BALB/c) in EMBL3 SP6/T7 phage (CLONTECH, Palo Alto, CA) was screened with a full-length cDNA probe for dectin-2 (Clone 1B12). Approximately 1 × 108 plaques were transferred onto nylon membranes and hybridized in buffer (50% formamide, 5× Denhardt's solution, 5× SSPE, 0.1% SDS, 100 mg/ml denatured salmon sperm DNA) containing the probe labeled with [α-32P]dCTP by Mega prime DNA labeling system (Amersham Pharmacia Biotech). Following a 16-h incubation at 42 °C, membranes were washed extensively in 2x SSC, 1% SDS, and then in 0.1x SSC, 0.1% SDS. The membranes were autoradiographed at 80 °C. Hybridized clones were isolated and further purified by repeating the screening. Finally, four independent phage clones were isolated.

**Phage DNA was purified from plate lysates of phage-infected E. coli and subcloned into a plasmid vector. The plate lysate was centrifuged at 8000 × g for 10 min and treated with 1 mg/ml of DNase I and 5 mg/ml of RNase A and followed by chloroform extraction. Phage particles were precipitated for 1 h at 4 °C with 10% polyethylene glycol (6000) and 1 M NaCl, and the pellet was resuspended in buffer (100 mM NaCl, 10 mM MgSO4, 35 mM Tris-HCl (pH 7.5), 2% gelatin) and extracted with phenol and chloroform. Finally, the phage DNA was precipitated with ethanol and dissolved in TE buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA).**

Genomic DNA fragments were excised from the phage DNA by digestion with BamHI and subcloned into a plasmid vector, pGEM-7zf+ (Promega, Madison, WI). To determine restriction fragments containing exons, the subcloned DNA was digested by different restriction enzymes and set southern hybridized with a 1B12 cDNA probe. The hybridized DNA fragments were cloned into a phagemid plasmid vector (Promega, Madison, WI) and then transferred into E. coli strain LMl (sce-2, thi-1, hsdR515), and the resulting phage clones were selected and expanded.

**RESULTS**

**Cloning of Dectin-2 cDNA and Its Deduced Amino Acid Sequence**—As described in the Introduction, using a subtractive cloning strategy (XS52 DC line minus J774 macrophage line), we isolated five cDNA clones that were expressed selectively by XS52 DC and that encoded novel polypeptides. One of these clones (clone 1C11–5) encoded a 244-amino acid polypeptide, termed dectin-1, which contained a single CRD motif in the COOH terminus of the molecule. Another clone 1B12 contained 630 nt in its open reading frame. As shown in Fig. 1, its deduced amino acid sequence revealed a 209-aa polypeptide of type II membrane-integrated configuration, consisting of a cytoplasmic domain (aa 1–14), a putative transmembrane domain (aa 15–42), and an extracellular domain (aa 43–209). The overall amino acid sequence encoded by clone 1B12 showed 33.5% sequence identity (Clustal method analyzed with Lasergene Program, DNA Star, Madison, WI) to murine (m) DCIR, a DC-associated surface receptor (21). The 1B12 polypeptide sequence also showed significant homology with...
other molecules, including mMGL (23.4%) (28), mHL2 (22.0%) (29), mHL1 (21.1%) (30), mCD69 (16.6%) (31), and mCD23 (16.3%) (32). Each of these molecules contained a single CRD motif at the COOH-terminal end and, thus, belongs structurally to the type II membrane-associated C-type lectin family. As indicated by asterisks in Fig. 1, the COOH-terminal region (aa 79–209) of the 1B12 polypeptide contained all the 13 invariant amino acid residues known to be conserved in the CRD motifs of many C-type lectins (33). Thus, we designated this polypeptide as DC-associated C-type lectin-2 or dectin-2.

As noted in Fig. 2A, the CRD domain in the dectin-2 polypeptide exhibited marked homology with the CRD sequences in other C-type lectins, such as DCIR (44.7%), MGL (43.8%), HL2 (45.8%), HL1 (39.6%), CD69 (33.3%), and CD23 (28.0%). The degree of sequence homology between dectin-1 and dectin-2 was 19.6% in the overall sequence and 24.8% within the CRD domain (Fig. 2B). Phylogenetic analysis of the CRD domains (by using the MegAlign function of Lasergene Program) indicated that dectin-2 is intermediate in structure between the HL/MGL subfamily and the CD23/CD69 subfamily, whereas dectin-1 appears to be further distant from either subfamily (Fig. 2C).

Like many C-type lectins (e.g. CD94, Ly-49, and NKG2) that are encoded in the natural killer gene complex (18, 34, 35), DCIR contains the consensus immunoreceptor tyrosine-based inhibitory motif (I/V)X(Y/A)X(L/V) in the cytoplasmic domain (21). Like other C-type lectins (e.g. CD23 and MGL), dectin-1 contained a putative immunoreceptor tyrosin-based activation motif (YXXL) (35, 36) in the cytoplasmic domain.

Tissue and Cell Distributions of Dectin-2 Transcripts—In Northern blotting, we identified a major dectin-2 mRNA of 1.5 kb expressed by the XS52 DC line (Fig. 3A). Dectin-2 mRNA was undetectable in any of the tested non-DC lines, including two macrophage lines (J774 and Raw), three T cell lines (7–17, HDK-1, and D10), a B cell hybridoma (5C5), a keratinocyte line (Pam 212), and a fibroblast line (NS01). The observed cell type specificity was not simply, because of the fact that only the XS52 DC line was cultured in the presence of granulocyte/macrophage-colony stimulating factor and CSF-1 (3, 4), because this line continued to express dectin-2 mRNA even after removal of these cytokines from culture medium and because its expression was not inducible in the J774 macrophage line by exposure to either cytokine (Fig. 3B). As shown in Fig. 3C, dectin-2 mRNA was expressed most abundantly in the spleen and thymus, tissues known to contain relatively large numbers of DC (1, 2).

Unexpectedly, dectin-2 mRNA was not detectable by Northern blotting in the skin, despite the facts that the XS52 DC line was established from this tissue (3) and that this DC line...
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resembles skin resident DC (Langerhans cells) in many features (3–13). We then tested dectin-2 mRNA expression in the epidermis using a more sensitive, RT-PCR analysis. As shown in Fig. 3D, two dectin-2 mRNA species were clearly detected in epidermal cells freshly isolated from BALB/c mice. The upper band represented the PCR product with the predicted molecular size of 631 base pairs, whereas the identity of the lower band remained unclear at that time (see below). Mouse epidermis contains keratinocytes and resident γδ T cells, in addition to Langerhans cells expressing the Ia (MHC class II) molecules. Importantly, depletion of the Ia+ epidermal cell population abrogated almost completely both PCR signals for dectin-2. This corroborates our observations with cell lines; dectin-2 mRNA was detected by Northern blotting in the Langerhans cell-like XS52 line, but was totally absent from the Pam 212 keratinocyte line and the 7–17 epidermal γδ T cell line (Fig. 3A). Thus, dectin-2 transcripts are expressed constitutively in the epidermis, and skin resident DC appear to be the major source of this expression. It should be emphasized that the observed expression patterns of dectin-2 mRNA were almost indistinguishable from those for dectin-1 mRNA.2

Identification of Dectin-2 Proteins—To raise anti-dectin-2 mAb, we prepared a fusion protein consisting of a 6x His tag and the extracellular domains (aa 46–209) of dectin-2. This fusion protein, His-dectin-2, was produced in E. coli, extracted in 8 M urea, and purified by nickel affinity chromatography. One of the rat mAb (R4C2) raised against His-dectin-2 recognized multiple bands (ranging from 23 to 31 kDa) in the whole cell extracts prepared from the XS52 DC line (Fig. 4A). Consistent with the predicted molecular structure as shown in Fig. 1, these bands were detected primarily in the membrane fraction isolated from the XS52 DC line (Fig. 4B). Most importantly, none of these bands were detected in the extracts prepared from any of the tested non-DC lines, including macrophage, B cell hybridoma, T cell, keratinocyte, and fibroblast lines (Fig. 4A). The two bands (of about 50 and 25 kDa) observed in the extracts from the 29B cell hybridoma represented immunoglobulin heavy and light chains being detected by our secondary Ab against rat IgG, because the same bands were also detected with the secondary Ab alone. Thus, dectin-2 proteins are produced selectively by the XS52 DC line, corroborating our observations at mRNA levels (Fig. 3A).

It was of our particular interest to determine whether His-dectin-2 proteins would recognize one or more conventional carbohydrate moieties. Soluble fractions of His-dectin-2 fusion proteins (containing the CRD motif) were labeled with125I and tested for the binding to agaroose beads that had been conjugated with mannose, fucose, lactose, GluNAc, or GalNAc. His-dectin-2 failed to exhibit specific binding to any of these carbohydrate probes, as determined by counting the radioactivities that were eluted by adding the corresponding carbohydrates (data not shown).

Identification of Two Truncated Isoforms of Dectin-2—As noted previously, we detected significant molecular heterogeneity in RT-PCR analysis (Fig. 3D) and immunoblotting (Fig. 4, A and B), suggesting the presence of different dectin-2 isoforms. This possibility was then tested by RT-PCR analyses using different sets of primers designed to amplify different regions of the dectin-2 gene. As shown in Fig. 5A (left panels), the primer set 3 (designed to amplify the entire coding se-

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**Fig. 3. Cell and tissue-specific expression of dectin-2 mRNA.** Total RNAs were isolated from XS52 DC, J774 and Raw macrophages, 7–17 DETC, HDK-1 Th1 cells, D10 Th2 cells, 5C5 B cell hybridoma, Pam 212 keratinocytes, and NS01 fibroblasts (A) or from the indicated tissues in adult BALB/c mice (C). B, XS52 cells and J774 cells were cultured for 48 h in the presence or absence of granulocyte/macrophage-colony stimulating factor (GM-CSF) (10 ng/ml) or CSF-1 (10 ng/ml) before RNA isolation. These RNA samples (10 μg/lane) were then examined by Northern blotting for dectin-2 or GAPDH. D, epidermal cells isolated from adult BALB/c mice were examined for dectin-2 mRNA expression by RT-PCR using the primer set 0 (“Experimental Procedures”) designed to amplify nt 475–1106. Some samples were treated with anti-Ia mAb plus complement to deplete Langerhans cells; the extent of depletion was assessed by measuring IL-1β mRNA, which is known to be expressed exclusively by Langerhans cells within murine epidermal cells.

**Fig. 4. Dectin-2 protein expression.** A, whole cell extracts were prepared from the indicated cell lines in the cell lysis buffer containing 0.3% Triton X-100. These samples were examined by immunoblotting with anti-dectin-2 mAb (R4C2) (top) and by Coomassie Blue staining for protein profiles (bottom). The data shown are representative of two independent experiments. B, crude lysates prepared from the indicated numbers (×10^5 cells/sample) of XS52 DC were centrifuged at 100,000 × g for 40 min, and the pellets (membrane fractions) were tested for the presence of dectin-2. Data shown are representative immunoblots with anti-dectin-2 mAb (R4C2) (top) and with control rat IgG (bottom) from three independent experiments.

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were used for PCR (from the 1B12), isoform b (encoded by clone 1E4). Their deduced amino acid sequences showed that the deletion of nt 263–362 in clone 1A7 and nt 569–691 in clone 1E4 with truncation within the CRD domain. As shown in Fig. 7, the full-length dectin-2 clone 1B12 exhibited two bands of 28 and 24 kDa after transfection with clone 1B12. By contrast, we detected two bands of 28 and 24 kDa after transfection with clone 1A7. Likewise, clone 1E4 produced two proteins with different molecular masses of 31 kDa, (b) β isoform with a 34 aa deletion in the neck domain (migrating as 28 and 24 kDa), and (c) γ isoform with 41 aa deletion in the CRD (migrating as 26 and 23 kDa). Mechanisms by which a single cDNA clone (encoding the β or γ isoform) produced two proteins with different migration profiles remain unclear at this moment.

Genomic Analysis of Dectin-2 DNA—To determine mechanisms for the generation of three transcripts encoding different isoforms, we cloned and sequenced genomic DNA fragments (14 kb in total) of dectin-2, covering from the cytoplasmic domain to the CRD domain. As shown in Fig. 7, the full-length dectin-2 isoform was found to be encoded by six exons, with exon 1 encoding 7 aa in the cytoplasmic domain, exon 2 encoding 31 aa mainly in the transmembrane domain, exon 3 encoding 34 aa

![Figure 5](image1.png)

**Fig. 5. Identification of truncated dectin-2 transcripts.** A, three primer sets designed to amplify the indicated regions of dectin-2 cDNA were used for PCR (top). Boxes with different patterns indicate, from the left, the intracellular, transmembrane, neck, and CRD domains of dectin-2. Total RNAs prepared from the XS52 DC line or from BALB/c mouse spleens were reverse-transcribed and then amplified with the indicated primer sets (left panels). Alternatively, dectin-2 cDNA clones (1B12, 1A7, and 1E4 from the left) were PCR amplified with the indicated primer sets (right panels). Data shown are the PCR products after 30 cycles of amplification. B, the deduced amino acid sequences of isoform α (encoded by clone 1B12), isoform β (encoded by clone 1A7), and isoform γ (encoded by clone 1E4) are shown as diagrams, with boxes with different patterns indicating, from the left, the intracellular, transmembrane, neck, and CRD domains of dectin-2.

![Figure 6](image2.png)

**Fig. 6. Identification of different dectin-2 isoform proteins.** COS-1 cells were transfected with clone 1B12 (isoform α), clone 1A7 (isoform β), clone 1E4 (isoform γ), or vector alone. Membrane fractions prepared from these transfected COS cells were examined for dectin-2 proteins by immunoblotting with anti-dectin-2 mAb (R4C2). The data shown are representative of three independent experiments.
mainly in the neck domain, and exons 4–6 encoding the CRD domain. The donor site (5′(C/A)AG-GUA/G)AGU and the acceptor site (5′(U/C)11N(U/C)AG(-G/A)) (37) were both conserved in each exon-intron interface. Remarkably, exon 3 was entirely skipped from the nucleotide sequence of clone 1A7. Interestingly, exons 5 and 6 were partially deleted in clone 1E4, and we identified the consensus motifs for splicing donor and acceptor sites within exon 5 and exon 6 at both ends (nt 569 and 691) where the 123-nt deletion was identified in clone 1E4. Based on these observations, we concluded that dectin-2 β and γ isoforms are generated through alternative splicing.

DISCUSSION

In the present study, we have identified a novel, DC-associated C-type lectin, termed dectin-2, which shared several important features with dectin-1. First, both dectin-2 and dectin-1 exhibited a common domain structure, consisting of a relatively short cytoplasmic domain, a transmembrane domain, an extracellular domain, and a single CRD in the COOH terminus. Second, dectin-2 mRNA expression profiles were indistinguishable from those for dectin-1 mRNA; that is, both were expressed: (a) at relatively high levels in the XS52 DC line but not in other tested non-DC lines, (b) most abundantly in spleen and thymus, and (c) constitutively in the Ia c and constitutively in the Ia a in other tested non-DC lines, (d) most abundantly in spleen and thymus, and (e) constitutively in the Ia a at relatively high levels in the XS52 DC line but not in other tested non-DC lines. Thus, dectin-2 and dectin-1 represent two structurally independent, DC-associated C-type lectins.

As described in the Introduction, DC have been shown to express both type I surface lectins (DEC-205 and MMR) and type II surface lectins (CD23, CD69, DCIR, and dectin-1). The present study adds three new members (dectin-2 α, β, and γ isoforms) to this list of DC-associated C-type lectin family. The dectin-2 α isoform showed the highest degree of homology (33.5% identity in the overall sequence and 44.8% within the CRD motif) to murine DCIR, which was identified recently by Bates et al. (21) by searching nucleotide data bases with a consensus sequence derived from the CRD motifs of HL-1, HL-2, and MGL. Dectin-2 differs from DCIR in that the immunoreceptor tyrosine-based inhibitory motif found in the intracellular domain of DICR was absent from the relatively short intracellular domain (14 aa) of dectin-2. Interestingly, dectin-2 also lacked an immunoreceptor tyrosine-based activation motif, which was identified in the intracellular domain of dectin-1. Therefore, an oversimplified scenario would be that dectin-1 and DCIR deliver counteracting signals into DC, whereas dectin-2 has no apparent signaling potential. Unfortunately, no information is available with respect to the natural ligands that are recognized by CD69, DCIR, dectin-1, and/or dectin-2. In this regard, none of the tested carbohydrate probes showed specific binding to His-dectin-2 (or to His-dectin-1). We interpreted these results to suggest that dectin-2 may recognize rather unique carbohydrate moieties or that dectin-2 may have to form heterodimers with a second C-type lectin (perhaps dectin-1) to exert high affinity carbohydrate binding, as has been shown for other type II lectins with single CRD motifs (38, 39).

One even more extreme possibility is that dectin-2 may recognize polypeptide or glycopolypeptide ligand(s). CD23 has been shown to recognize a polypeptide motif, instead of carbohydrate residues, of the lGε molecule (40), documenting that carbohydrates do not necessarily serve as natural ligands of all the molecules that “structurally” belong to the C-type lectin family. Further studies are required to identify the ligands recognized by the DC-associated type II surface lectins (including dectin-2) and to determine their physiological functions.

Production of different isoforms by alternative splicing has been reported for other members of the type II surface lectin family. For example, Nunez et al. (42) identified a truncated transcript of CD23 from which exon 3 (encoding the transmembrane domain and a portion of the cytoplasmic domain) was deleted by alternative splicing. It was postulated that the resulting CD23 isoform lacking the transmembrane domain might serve as a soluble CD23 receptor controlling CD23-mediated immunological function. Ying et al. (43) identified several alternatively spliced transcripts lacking exon 3 (encoding the cytoplasmic tail) and/or exon 4 (encoding the transmembrane domain) of CD72, a type II surface lectin expressed primarily by B cells. More recently, Furukawa et al. (44) identified an alternatively spliced transcript lacking exon 2 (encoding the transmembrane domain) of CD94, one of the natural killer-associated type II surface lectins. In this regard, the isoforms we identified in dectin-2 are unique in that they lack the neck domain or a portion of the CRD motif. Thus, our observation provides additional mechanims for creating molecular heterogeneity of type II surface lectins.

In summary, we postulate that dectin-2 and its isoforms, together with other molecules (CD23, CD69, DCIR, and dectin-1), may form a unique subfamily of DC-associated, type II surface lectins. The present study provides an important piece of information for revealing the potential functions of those DC-associated molecules.

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