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Dendritic Cell-Associated Lectin-1: A Novel Dendritic Cell-Associated, C-Type Lectin-Like Molecule Enhances T Cell Secretion of IL-4

Elizabeth J. Ryan,*‡ Aaron J. Marshall,**§ Dario Magaletti,‡ Helen Floyd,**† Kevin E. Draves,† N. Eric Olson,* and Edward A. Clark*‡‡

We have characterized dendritic cell (DC)-associated lectin-1 (DCAL-1), a novel, type II, transmembrane, C-type lectin-like protein. DCAL-1 has restricted expression in hematopoietic cells, in particular, DCs and B cells, but T cells and monocytes do not express it. The DCAL-1 locus is within a cluster of C-type lectin-like loci on human chromosome 12p12–13 just 3′ to the CD69 locus. The consensus sequence of the DCAL-1 gene was confirmed by RACE-PCR; however, based on sequence alignment with genomic DNA and with various human expressed sequence tags, we predict that DCAL-1 has two splice variants. C-type lectins share a common sequence motif of 14 invariable and 18 highly conserved aa residues known as the carbohydrate recognition domain. DCAL-1, however, is missing three of the cysteine residues required to form the standard carbohydrate recognition domain. DCAL-1 mRNA and protein expression are increased upon the differentiation of monocytes to CD1a+ DCs. B cells also express high levels of DCAL-1 on their cell surface. Using a DCAL-1 fusion protein we identified a population of CD4+ CD45RA+ T cells that express DCAL-1 ligand. Coincubation with soluble DCAL-1 enhanced the proliferation of CD4+ T cells in response to CD3 ligation and significantly increased IL-4 secretion. In contrast, coincubation with soluble DC-specific ICAM-3-grabbing nonintegrin (CD209) fusion protein as a control had no effect on CD4+ T cell proliferation or IL-4 and IFN-γ secretion. Therefore, the function of DCAL-1 on DCs and B cells may act as a T cell costimulatory molecule, which skews CD4+ T cells toward a Th2 response by enhancing their secretion of IL-4. The Journal of Immunology, 2002, 169: 5638–5648.

C-type lectins bind sugars in a calcium-dependent manner using highly conserved carbohydrate recognition domains (CRDs). These CRDs contain calcium-binding pockets that are essential for carbohydrate ligand binding (1). In addition, C-type lectin-like receptors have now been characterized, and these consist of a CRD-like domain that might bind protein or lipids rather than carbohydrates (2). There are two groups of membrane-bound, C-type lectins, distinguished on the basis of the orientation of their amino (N) terminus. Type I C-type lectins have extracellular N termini, while type II have cytoplasmic N termini. The type I surface lectins that have been characterized have multiple CRDs, while the type II C-type lectins identified to date have one CRD or CRD-like domain (2).

Dendritic cells (DCs) are the most effective APC for initiating primary immune responses. Peripherally located immature DCs capture Ags via pinocytosis or receptor-mediated endocytosis, and Ag processing occurs in endosomes before loading the antigenic peptides into MHC class II molecules for presentation to T cells. During this process, DCs migrate to the T lymphocyte areas of draining lymph nodes and differentiate functionally and phenotypically from an immature state to a more activated state for Ag presentation (3, 4). Immature DCs lack the cell surface markers found on mature DCs, e.g., CD83, and express low levels of the costimulatory molecules, CD40, CD80, and CD86 (4). After exposure to inflammatory stimuli, DCs lose their Ag-processing capacity (5, 6). During this functional transition, DCs increase the expression of MHC class II, CD40, CD80, and CD86 and alter their expression pattern of chemokine receptors.

DCs express surface receptors, e.g. the Toll-like receptors, that recognize pathogen-associated molecular patterns, such as bacterial LPS, unmethylated CpG motifs of bacterial DNA, double-stranded viral RNA, and yeast mannans (7). Recently, a number of reports have shown that many type II, C-type lectins are expressed by DCs including, DC lectin (DLEC), DC immunoreceptor (DCIR), dectin-1, dectin-2, C-type lectin receptor (CLEC), asialoglycoprotein receptor, CIRE, and DC-specific ICAM-3-grabbing nonintegrin (SIGN) (8–17). The function of most of these receptors remains to be elucidated. However, it is becoming more apparent that this family of receptors serves multiple functions in the immune system by mediating both pathogen recognition and cell-cell interactions (17). For example, DC-SIGN can interact with the glycoprotein gp120 of HIV and the adhesion molecules CD50 and CD104 (15, 16). Another C-type lectin, dectin-1, a β-glucan receptor, can bind pathogenic yeast and also T cells (18, 19). The type I, C-type lectin, the macrophage mannose receptor, is a cell surface protein that can mediate the phagocytosis of microorganisms (6). DEC-205 (CD205), another DC-associated type I, C-type lectin may also interact with pathogens and mediate Ag uptake (20). Therefore, it is likely that other C-type lectin receptors expressed on DCs play important roles in the recognition of
pathogens and the activation of host defense pathways controlling and coordinating innate and adaptive immune responses.

To identify genes expressed in human germinal centers (GCs), we used subtractive hybridization with tester cDNA derived from tonsillar cells enriched for follicular dendritic cells (FDCs) (21). One of the isolated clones encoded for a novel type II C-type-like lectin molecule, DC-associated lectin-1 (DCAL-1). DCAL-1 is expressed most abundantly in DCs and GC B cells, but is not expressed in T cells. To further elucidate the function of DCAL-1 in the immune system, we determined its expression pattern in immune cells. Interestingly, DCAL-1 mRNA expression is up-regulated in tonsillar B cells by CD40 stimulation. Furthermore, DCAL-1 mRNA and protein expression are up-regulated upon the differentiation of monocytes to immature DCs, and this expression is maintained upon maturation of DCs with LPS or CD40 stimulation. In addition, we have used a DCAL-1 histidine (His)-tagged fusion protein in parallel with a DC-SIGN His-tagged fusion protein to identify cells expressing potential DCAL-1 or DC-SIGN ligands. We found that both of these proteins bound to subsets of T cells, and that DCAL-1, unlike DC-SIGN, acts as a costimulatory molecule to enhance IL-4 production by CD4+ T cells.

Materials and Methods

Isolation of DCAL-1 cDNA

We isolated RNA from human tonsillal cell populations enriched for FDCs and then conducted a suppression subtractive hybridization-PCR procedure as previously described (21). One clone isolated in this way encoded a novel C-type-like lectin, DCAL-1. A putative translational start site upstream of the transmembrane region was identified in a putative exon in the genomic sequence (human bacterial artificial chromosome [BAC] clone RPCI11-751L, accession no. AC007068) using the program GRAIL (Accelrys, San Diego, CA) (22).

The sequences for the transmembrane and cytoplasmic regions were obtained by 3’- and 5’-RACE PCR using the SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA) with cDNA isolated from CD40 ligand (CD40L)-stimulated tonsillar human B cells. This sequence was confirmed by PCR using cDNA obtained from tonsillar B cells stimulated for 24 h with CD40L (Alexis Biochemicals, San Diego, CA) using the following primers: 5’-aaaacgagaacctactgtatgg-3’ and 5’-ctcaaggttggatccaatcaaaactgttcggacttccccg-3’. The resulting product was cloned into the PCR 2.1 vector using the QIAexpress kit (Qiagen, Chatsworth, CA). The six-His-tagged DCAL-1 and six-His-tagged DC-SIGN were produced by inserting the DC-SIGN-His, and binding was detected by the six-His-specific antibody. The sequences for the transmembrane and cytoplasmic regions were obtained by 3’- and 5’-RACE PCR using the SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA) with cDNA isolated from CD40 ligand (CD40L)-stimulated tonsillar human B cells. This sequence was confirmed by PCR using cDNA obtained from tonsillar B cells stimulated for 24 h with CD40L (Alexis Biochemicals, San Diego, CA) using the following primers: 5’-aaaacgagaacctactgtatgg-3’ and 5’-ctcaaggttggatccaatcaaaactgttcggacttccccg-3’. The resulting product was cloned into the PCR 2.1 vector using the QIAexpress kit (Qiagen, Chatsworth, CA). The six-His-tagged DCAL-1 and six-His-tagged DC-SIGN were produced by inserting the DC-SIGN-His, and binding was detected by the six-His-specific antibody.

We hybridized a 32P-labeled cDNA probe corresponding to aa 57–105 of the short splice variant of DCAL-1 (Fig. 1A) to human multiple tissue Northern Blot (Clontech, Palo Alto, CA) and to a Northern blot of 2 μg of total RNA from human tonsils, according to the manufacturer’s protocol.

RT-PCR expression analysis

Monocytes were isolated from peripheral blood of healthy donors (American Red Cross, Portland, OR) using positive selection with anti-CD14-labeled magnetic beads (Miltenyi Biotec, Auburn, CA); the purity of the monocytes obtained was routinely ≥95%. CD14+ cells were differentiated into CD1a+ cells in the presence of GM-CSF (Immunex, Seattle, WA) and IL-4 (Research Diagnostics, Flanders, NJ) for 5–7 days. The cells were matured to CD83+ cells by incubating with mAb to CD40 (G28-5) or Escherichia coli LPS (Sigma, St. Louis, MO) for 24 or 72 h. Human tonsillar B cells were prepared as previously described (24). BDCA-2+ and CD1c+ DCs were isolated from peripheral blood by positive selection with BDCA-2- and CD1c-labeled magnetic beads (Miltenyi Biotec). The purified primary cells or cell lines were lysed in TRIzol reagent (Life Technologies/Invitrogen, Carlsbad, CA), and RNA was isolated as described by the manufacturer. First-strand cDNA was synthesized using random hexamers and avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). DCAL-1 expression was analyzed by PCR of the cDNA in 10-fold dilutions using the following DCAL-1-specific primers: 5’-gtaggagttgtggagagatggcggagtctcgc-3’ and 5’-ctgccgatgggagatggtagctgcg-3’. The following G3PDH primers were used as positive controls: 5’-accagcattcagcataac-3’ and 5’-ttcaccacacctgttctga-3’.

DCAL-1-His/DC-SIGN-His protein production

The six-His-tagged DCAL-1 and six-His-tagged DC-SIGN were produced using the QIAexpress kit (Qiagen, Chatsworth, CA). The following primers were used to make the expression constructs using the pQE-31 vector: DCAL-1 (BanHI), 5’-gtgtggtgacataaacataaaagcaactctgccc-3’; DCAL-1 (HindIII), 5’-cgcaagttgtcttgaactaatctatgttg-3’; and for DCSIGN, the PQE-30 vector DC-SIGN (BanHI), 5’-gtgtggtgacataaacataaaagcaactctgccc-3’; and DC-SIGN (HindIII), 5’-cgcaagttgtcttgaactaatctatgttg-3’; and for DC-SIGN, the PQE-30 vector DC-SIGN (BanHI), 5’-gtgtggtgacataaacataaaagcaactctgccc-3’; and DC-SIGN (HindIII), 5’-cgcaagttgtcttgaactaatctatgttg-3’. The constructs were transfected into E. coli M15 (pREP4), the bacteria were grown in Luria–Bertoni broth supplemented with ampicillin (100 μg/ml) and kanamycin (25 μg/ml). Protein expression was induced with 1 mM isopropyl-β-D-galactopyranoside. The proteins were solubilized in urea, purified by chromatography on a nickel-nitrilo-triacetic acid resin column (Qiagen) as directed by the manufacturer, and then refolded by stepwise dialysis as described previously (25).

Both the DCAL-1 and DC-SIGN fusion proteins, consisting of the extracellular portion of the molecules including the CRD as a stop codon, were inserted in the transmembrane region. The DC-SIGN fusion protein is composed of the extracellular portion of DC-SIGN (CD209), which was reported by Curtis et al. (26). In a direct ELISA, a DC-SIGN-specific mAb (DCN46, BD PharMingen) recognized the DC-SIGN fusion protein. At 10 μg/ml both fusion protein preparations were negative for LPS when tested by a Limulus amoebocyte lysate gel clot assay (Sigma).

mAb production

BALB/c mice were injected i.p. with either DCAL-1-His or DC-SIGN-His formulated with monophosphoryl lipid A, and trehalose dicorynomycolate emulsion (Corixa, Hamilton, MT) as adjuvant. Mice were boosted at wk 2 and 10. Three days following the final boost spleens were removed, and hybridomas were made by fusion with NS-1 cells. An ELISA screen using the DCAL-1 and DC-SIGN proteins was used to determine positive clones. False positives, i.e., any sample positive for both DCAL-1 and DC-SIGN, were eliminated. One clone, UW50 (IgM), was positive for DCAL-1 and negative for DC-SIGN in the ELISA screen, and on further subcloning retained these characteristics.

Cell surface expression of DCAL-1

Various primary cells and cell lines were incubated with the DCAL-1-mAb (UW50) or the isotype control (mouse IgM), followed by rat anti-mouse IgM-FITC (BioSource, Camarillo, CA). CD1a+ DCs and dense tonsillar B cells were also incubated for 24 h with 1 μg/ml soluble recombinant CD40L (Alexis Biochemicals). DCAL-1 protein expression on the cell surface was then determined by flow cytometry on a FACScan analyzer (BD Biosciences, Mountain View, CA).

DCAL-1/DC-SIGN binding assays

PBMC from healthy donors were incubated with 10 μg of DCAL-1-His or DC-SIGN-His, and binding was detected by the six-His-specific biotinylated Ab (Berkeley Antibody, Richmond, CA); at 10 μg/ml, followed by streptavidin-PerCP (BD Pharmingen). In some experiments cells were also stained for different lineage markers with FITC- and PE-conjugated Abs (BD Pharmingen): CD14-FITC, CD11c-PE, CD122-FITC (1F5), CD19-PC5 (J4.119), CD45RA-FITC (L48), CD45RO-PE (2H4), and CD20-FITC (4D6, BD PharMingen). DCAL-1/DC-SIGN binding was detected by the six-His-specific antibody. In some experiments cells were also stained for different lineage markers with FITC- and PE-conjugated Abs (BD Pharmingen): CD14-FITC, CD11c-PE, CD122-FITC (1F5), CD19-PC5 (J4.119), CD45RA-FITC (L48), CD45RO-PE (2H4), and CD20-FITC (4D6, BD PharMingen). DCAL-1/DC-SIGN binding was detected by the six-His-specific antibody.

T cell costimulation assays

Total CD3+ T cells were isolated from PBMC by SRBC agglutination (RBCs from Triple J Farms, Bellingham, WA). The CD3+ cells isolated

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were routinely >97% pure. CD4+ T cells were isolated by panning with CD8 mAb OKT4 for 1 h at 37°C. CD4+ and CD8+ T cells were isolated by panning with CD4 mAb OKT4 for 1 h at 37°C. CD4+ and CD8+ T cells were purified by negative selection using anti-CD45RO-labeled microbeads (Miltenyi Biotech, Auburn, CA). CFSE (Molecular Probes, Eugene, OR) labeling of CD4+ and CD8+ T cells was performed as previously described (27). Total T cells or CD4+ and CD8+ T cells were cocultured with CD3 mAb (64.1) at 1, 0.3, 0.1, and 0 µg/ml (in solution); with medium alone; or with 0.5, 1, 5, 10, and 25 µg/ml of DC-SIGN fusion proteins for 5 days at 37°C in 5% CO2. In some experiments total T cells or CD4+ and CD8+ T cells were cocultured with CD3 (64.1) and CD28 (9.3) mAbs (both at 1 µg/ml in the absence or the presence of 0.5, 1, 5, 10, and 25 µg/ml of DC-SIGN fusion proteins. All cells were cultured in complete RPMI 1640 with 10% FCS and 50 µM 2-ME. We assessed the number of cell divisions by analyzing CFSE dye extinction with a FACScan. Supernatants were removed and frozen at −20°C for cytokine analyses.

Allogenic MLR

MLRs were set up by culturing different concentrations (1,500–50,000/well) of gamma-irradiated MDDCs (3,000 rad of 137 Cs) with 10,000/well of CD4+ and CD8+T cells. These cells were cocultured in 96-well, round-bottom microtiter plates in a final volume of 200 µl for 3 days with soluble DC-SIGN or DC-SIGN fusion proteins for 5 days at 37°C in 5% CO2. In some experiments total T cells or CD4+ and CD8+T cells were cocultured with CD3 (64.1) and CD28 (9.3) mAbs (both at 1 µg/ml) in the absence or the presence of 0.5, 1, 5, 10, and 25 µg/ml of DC-SIGN fusion proteins. All cells were cultured in complete RPMI 1640 with 10% FCS and 50 µM 2-ME. We assessed the number of cell divisions by analyzing CFSE dye extinction with a FACScan. Supernatants were removed and frozen at −20°C for cytokine analyses.

Cytokine analysis

We analyzed IL-4 and IFN-γ secretion by capture immunoassay in triplicate using matched pairs of cytokine-specific mAbs: capture anti-IL-4, 8D4-8; detection anti-IL-4, MP4-25D2; capture anti-IFN-γ, 4S.B3 (BD PharMingen). Concentrations of cytokines were extrapolated from a standard curve prepared with recombinant cytokine (BD PharMingen). Detection limits of the assay were 15 pg/ml for IL-4 and 1.25 ng/ml for IFN-γ. Cytokine production was also detected by intracellular cytokine staining as previously described (28). Cells were stimulated as described above, but for the last 6 h of culture, PMA (25 ng/ml; Sigma), ionomycin (100 ng/ml; Calbiochem), and Golgi-Stop (as recommended by the manufacturer, BD PharMingen) were added. Cells were then washed, fixed with 4% paraformaldehyde, permeabilized with 0.1% saponin/1% FCS/PBS, and labeled with anti-IL-4-PE (8D4-8; BD PharMingen), detection anti-IFN-γ, MP4-25D2; capture anti-IFN-γ, 4S.B3 (BD PharMingen), or IgG1-PE isotype control. The cells were analyzed by flow cytometry, and results were analyzed using CellQuest software (BD Biosciences).

Results

DCAL-1 gene discovery

To identify genes expressed in human germinal centers (GCs), we used subtractive hybridization with tester cDNA derived from ton- silar cells enriched for FDCs (21). One of the isolated clones encoded for a novel C-type lectin, DCAL-1. Further sequence was obtained using 5’- and 3’-RACE-PCR, and full-length DCAL-1 was cloned from human tonsillar B cells (accession no. AF518873). Overlapping EST clones encoding the DCAL-1 sequence were obtained by searching the human EST database, including a clone derived from a B cell library as well as a number of clones from prostate and testis carcinomas. The intron/exon structure of the DCAL-1 gene was deduced by alignment of the sequence and ESTs with the BAC clone RPC111-75L1 (AC007068) and by using the program GRAIL (22) (Fig. 1A). This analysis of the genomic sequence suggests that there are alterna-tively spliced forms of DCAL-1 (Fig. 1A). The first splicing site is in the neck region of the lectin, which changes the length of the molecule that extends from the cell surface, but not the intracel-lular domain or the C-type lectin domain (140533–140702; Fig. 1A). A similar splicing pattern has been observed in the human α-glucan receptor or dectin-1 (19). Sequence analysis of the BAC clone RPC111-75L1 indicates that the regions 144735–144928 and 146739–147394 (Fig. 1A) are also possible exons. Therefore, DCAL-1 may also be expressed with alternatively spliced C termini.

Chromosomal location and protein structure of DCAL-1

The EST search revealed that the DCAL-1 locus is within a cluster of C-type lectin loci on human chromosome 12p12–13 just 3’ to the CD69 locus (Fig. 1A). The gene complex on chromosome 12p12–13 encodes type II transmembrane proteins with a C-type lectin domain, which trigger or inhibit target cell lysis by NK cells (NKR-P1, Ly49, NKG2, CD94) or function as cellular activators of various hemopoietic cells (CD69) (1). Another gene closely linked to DCAL-1 is the activation-induced C-type lectin (AIr) which is expressed in cell types of hemopoietic origin including T cells, B cells, and monocytes (29). Macrophage galactose/N-acetylgalactosamine-specific C-type lectin (mMGL) is also closely associated with the DCAL-1 gene. mMGL is involved in immune responses directed against metastatic tumor cells (30). As these related genes have important functions in directing the immune response, we decided to study further the expression pattern and function of DCAL-1 in the immune system.

Analysis of the amino acid sequence of DCAL-1 revealed features typical of a type II transmembrane protein with a single extracellular CRD-like domain (Fig. 1C). The protein lacks a cleavable signal sequence and has a putative hydrophobic transmembrane region and two potential glycosylation sites. Comparison with protein sequence databases showed similarity in the CRD to C-type lectin receptors CD69 (62% similarity, 39% identity), NKR-P1, (49% similarity, 26% identity), DCIR, (57% similarity, 40% identity), and DLEC, (58% similarity, 41% identity; Fig. 1D). Phylogenetic analysis reveals that LTL1 and CLR-b are the most closely related molecules to DCAL-1 based on similarity in the CRD (data not shown). The alignment of the CRD of DCAL-1 with related C-type lectins suggests that the CRD of DCAL-1 is not a standard CRD, but, rather, a truncated form (Fig. 1D). For a complete CRD, six conserved cysteine residues, which generate three intrachain disulfide bonds, are required (2, 31). DCAL-1, however, possesses only three of these six conserved cysteines; therefore, from the amino acid sequence we propose that DCAL-1 generates one defined bond and possibly a second with an undefined cysteine outside of the CRD or possibly generates an interchain disulfide bond to form a dimer or multimer.

There is no obvious Ca2+ binding site present in the amino acid sequence, suggesting a Ca2+-independent function. This has been previously reported in NK lectin receptors, which bind to MHC class I ligands (1, 2, 32). The truncated CRD of DCAL-1 lacks the hydrophobic core or WIGL domain that is possessed by most related C-type lectins (Fig. 1D).

DCAL-1 gene expression is restricted to lymphoid tissue and hemopoietic cells

DCAL-1 has restricted expression in human tissues, as determined by Northern blot analysis (Fig. 2A). DCAL-1 is expressed in spleen, lymph node, and tonsil and at lower levels in peripheral blood, bone marrow, and colon, but not in thymus. Interestingly, EST clones encoding DCAL-1 were derived from prostate and testis carcinomas; however, no expression was detected in normal prostate and testis tissue (Fig. 2A). However, DCAL-1 mRNA was expressed by a number of B cell lines (Fig. 2B), and the levels of mRNA detected varied among the B cell lines tested. DCAL-1 mRNA was also highly expressed by primary human tonsillar B cells (Fig. 3C). While the expression of DCAL-1 mRNA is up-regulated following stimulation via CD40 (Fig. 3C), DCAL-1 protein expression, which is already highly expressed on the surface of resting cells, is not altered significantly (Fig. 4A). All B cell
lines tested to date, including BJAB (Fig. 4A) express high levels of DCAL-1 protein on their surface. In addition, DCAL-1 mRNA is not expressed in T cell lines, an epithelial cell line (HeLa) or the myeloid cell line, HL60 (Fig. 2B). The T cell line Jurkat and CD3+ T cells isolated from peripheral blood also do not express DCAL-1 protein on their surface (Fig. 4C).

There are different populations of DCs in peripheral blood: BDCA-2+ CD11c+ CD123bright plasmacytoid DCs and CD1c-

**FIGURE 1.** DCAL-1 gene structure and sequence. A, Schematic showing the structure of the human DCAL-1 gene, deduced by alignment of DCAL-1 cDNA sequence with the human BAC clone RPCI11-75L1 (accession no. AC007068) The regions 140533–140702, 144735–144928, and 146739–147394 represent possible splice variants of DCAL-1. B, Nucleotide sequence of DCAL-1. C, DCAL-1 amino acid sequence. The underlined area is the CRD with ~33% homology with standard CRD, the broken underline indicates the putative transmembrane region, and the asterisk represent putative glycosylation sites. D, Alignment of the DCAL-1 CRD protein sequence with related C-type lectin CRD. *Conserved residue; : conserved charge.
CD11c^{bright} CD123^{dim} myeloid DCs (33). By RT-PCR analysis DCAL-1 expression did not vary between the DC subpopulations (Fig. 2C). However, only the CD1c^{+} CD11c^{bright} CD123^{dim} subpopulation of DCs expressed high levels of DCAL-1 protein on their cell surface (Fig. 4D).

Resting CD14^{+} monocytes do not express DCAL-1 mRNA (Fig. 3A), or protein (Fig. 4B). However, differentiation of monocytes to CD1a^{+} immature DCs with GM-CSF and IL-4 up-regulated both DCAL-1 mRNA (Fig. 3B) and protein expression (Fig. 4B). Immature CD1a^{+} monocyte-derived DCs matured by either anti-CD40 or LPS, maintained expression of DCAL-1 (Figs. 3B and 4B).

Identification of the cell types expressing DCAL-1 ligand
To identify the cell types to which DCAL-1 binds, we incubated PBMCs with His-tagged DCAL-1 protein, and binding was detected by a biotin-anti-His Ab and streptavidin-PE. A DC-SIGN-His tagged fusion protein produced in the same way as DCAL-1 was used as a control protein. DC-SIGN binds to CD102 (ICAM-2) and CD50 (ICAM-3) (34). Both DCAL-1 and DC-SIGN bound a population of CD4^{+} CD45RA^{+} T cells (Fig. 5B). However, DCAL-1, but not DC-SIGN (data not shown), specifically bound a population of CD20^{+} peripheral blood B cells, but not dense tonsillar B cells (Fig. 5A). A small population of CD14^{+} cells also bound DCAL-1 and DC-SIGN, perhaps due to nonspecific phagocytosis of the fusion proteins (data not shown). The binding pattern of DCAL-1 to B cells and to a subset of T cells suggests that DCAL-1 may function in mediating immune cell-cell interactions.

DCAL-1 may act as a T cell costimulatory molecule to enhance Th2 immunity
Purified CD4^{+} T cells were labeled with CFSE and then stimulated in vitro with or without varying concentrations of anti-CD3 in the presence or the absence of DCAL-1 or the control DC-SIGN His-tagged fusion proteins. We initially assessed T cell proliferation by determining the extinction of the CFSE staining. Coincubation of T cells with the DCAL-1 fusion protein and anti-CD3 enhanced proliferation even at very low doses of soluble CD3 mAb (0.1 μg/ml). However,
at similar concentrations the DC-SIGN His-tagged fusion protein was less effective (Fig. 6). Incubation of T cells with the DCAL-1 or DC-SIGN fusion proteins alone had no effect on proliferation. To investigate whether coinubcation of T cells with DCAL-1 fusion protein and anti-CD3 had any effect on the type of T cell responses obtained, we measured cytokine production by cells that we have shown to bind to DCAL-1: CD4⁺/CD45RA⁺ T cells. DCAL-1 significantly increased the secretion of IL-4 by anti-CD3-stimulated T cells in a dose-dependent manner (Fig. 7A). The optimum dose of DCAL-1 to enhance IL-4 secretion was 10 μg/ml (Fig. 7A). However, DCAL-1 costimulation did not cause the T cell response to be completely polarized to Th2, as IFN-γ secretion was not inhibited (Fig. 7A). In contrast, coinubcation of T cells with anti-CD3 and DC-SIGN fusion protein did not affect the secretion of IL-4 or IFN-γ (Fig. 7A). Total T cells or CD4⁺CD45RA⁺ T cells were stimulated with a combination of anti-CD3 and anti-CD28 with or without the DCAL-1 or DC-SIGN fusion proteins (0.5, 1, 10, and 25 μg/ml), and both DCAL-1 and DC-SIGN fusion proteins had no additive costimulatory effect on CD3/CD28-induced proliferation or the polarization of cytokine secretion patterns, indicating that the costimulatory effect of
DCAL-1 is weaker than that of CD28 (data not shown). Costimulation with DCAL-1 fusion protein also enhanced the amount of IL-4 produced by T cells, as detected by intracellular cytokine staining, increasing the mean fluorescence intensity almost 2-fold over anti-CD3 stimulation alone (Fig. 7B).

Soluble DCAL-1 enhanced the stimulatory capacity of monocyte-derived DCs in an allogenic MLR (Fig. 8). Soluble DCAL-1 (10 μg/ml) significantly increased the proliferation of CD4<sup>+</sup>CD45RA<sup>+</sup> T cells when added with DCs at 5 × 10<sup>4</sup> cells/ml and 2.5 × 10<sup>5</sup> cells/ml (p < 0.001). Soluble DC-SIGN (10 μg/ml) also significantly increased the proliferation of CD4<sup>+</sup>CD45RA<sup>+</sup> T cells when added with DCs at 5 × 10<sup>4</sup> cells/ml (p < 0.05), but not to the same extent as the DCAL-1 fusion protein (Fig. 8). A dose of 10 μg/ml of soluble DCAL-1 or DC-SIGN was determined to be the optimum concentration. In a dose-response experiment where 0, 5, 10, and 25 μg/ml of the fusion proteins were included in an MLR, maximum proliferation was observed with the 10 μg/ml dose (data not shown).
Discussion

Here we have described a novel gene product, DCAL-1, which is highly expressed on DCs and B cells. The DCAL-1 locus is within a cluster of C-type lectin loci on human chromosome 12p12–13 just 3′ to the CD69 locus. This region contains a large number of genes that encode type II transmembrane proteins with C-type lectin and C-type-like lectin domains (1, 17). C-type lectins are proteins that share a common sequence motif, the CRD, with 14 invariant and 18 highly conserved aa residues (2, 31). These lectins were initially defined as proteins involved in the recognition of oligosaccharides in a Ca²⁺-dependent manner via their CRD (2, 31). A number of C-type lectins do not bind sugars due to a divergence from the prototype CRD (2). These divergent CRDs, known as C-type lectin-like domains, have some of the conserved residues required for establishing the C-type lectin fold, but not the residues required for carbohydrate recognition. CRDs to date have been shown to vary in length from 115–130 aa. The standard CRD expressed by lectins coded for in the NKC contains six invariant disulide-bonded cysteines (2, 31). DCAL-1, however, has a truncated CRD of 42 aa. Other lectin-like molecules also deviate from the standard CRD, such as the recently cloned Clr family of lectin-like genes localized between mouse NKRPl and CD69; the Clr lectin domain lacks the fifth cysteine (35). However, in some Clr molecules, the fourth cysteine that usually forms the disulide bridge with cysteine 5 is also missing (35). Another example of a lectin-like molecule with a truncated CRD is LLT1 (36); here the fifth cysteine is missing. DCAL-1 illustrates yet another way in which C-type lectins display molecular heterogeneity.

The cytoplasmic portion of the predicted amino acid sequence of DCAL-1 does not contain any signaling motifs, suggesting that to mediate intracellular signals DCAL-1 may have to form a heterodimer analogous to CD94/NKG2a (32). Alternatively, one intriguing hypothesis is that DCAL-1, through the charged residues

![Figure 7](http://www.jimmunol.org/)
in the transmembrane region, may associate with a molecule such as DAP12, an immunoreceptor tyrosine-based activation motif-bearing membrane adaptor molecule implicated in the activation of NK and myeloid cells (37).

DCAL-1 has possibly two splice variants (Fig. 1A). The two bands detectable by Northern blotting in spleen, PBL, tonsil, and lymph node may represent two alternatively spliced forms of DCAL-1 (Fig. 2A). The long form of DCAL-1 contains a stalk region separating the CRD from the transmembrane region. The human β-glucan receptor/dectin-1 (19) also has a similar splicing pattern with two stalk regions of varying length separating the transmembrane region from the CRD. While the significance of the alternative splice forms of DCAL-1 remains unclear, the alternate splice variants of dectin-1 do not have a significant effect on its ability to recognize β-glucans or yeast (19). One possibility is that the longer stalk region may enhance the molecule’s flexibility and allow it to project above other molecules on the cell membrane, therefore binding to its ligand more efficiently. The different isoforms may also have different regulatory roles, a phenomenon described for other cell surface receptors such as CD40 (38) and the scavenger receptor type A (39). Dectin-2, another recently described C-type-like lectin, which exhibited a similar expression pattern as dectin-1, also has multiple splice variants (11).

DCAL-1 protein is expressed strongly by both B cells, and DCs (Fig. 4, A and B). DCAL-1 mRNA, but not protein expression, is increased by CD40 stimulation of B cells (Fig. 3C). Both mRNA and protein expression is enhanced by the differentiation of monocytes to CD1a+ DCs (Figs. 3B and 4B). Activation of immature DCs by anti-CD40, or LPS stimulation maintains DCAL-1 mRNA and protein expression (Figs. 3B and 4B). This suggests that DCAL-1 may play a role in either Ag uptake or Ag presentation, as upon DC maturation the cells change phenotypically from cells specialized in Ag uptake, to mature cells with receptors for Ag presentation and T cell costimulation. DCAL-1 expression by DCs differs from that by other recently described DC-associated, C-type lectins, such as DCIR, which is down-regulated by DC maturation stimuli, such as LPS, TNF-α, and CD40L (9). The expression pattern of DCAL-1 on DCs also differs from that of CLEC-1, a C-type lectin homologous to the NKG2 family. CLEC-1 is only expressed by DCs that have received maturation signals via CD40-CD40L interaction or TNF-α (12). The DC-restricted C-type lectin, DC- asialglycoprotein is found only on immature DCs, with expression lost upon maturation of DCs by CD40 ligation (13). Another novel C-type lectin, CIRE, which shares 57% homology with DC-SIGN, is expressed at higher levels in myeloid-related murine CD8α+ DCs than in the lymphoid CD8α+ DCs (14).

Evidence is emerging that C-type-like lectins are expressed differentially by DC subsets (17). DC-SIGN is expressed on only a small subset of blood DCs (34). BDCA-2 is absent from monocyte-derived DCs and is specifically expressed by a subset of blood DCs, the CD11c−CD123 bright plasmacytoid DCs (33, 40). These cells secrete large amounts of type I IFN. In this study we show that while both CD11c− CD123 bright and CD11c+ CD11c bright CD123 dim subsets of DCs express DCAL-1 mRNA, but only the CD11c+ CD11c bright CD123 dim population expresses DCAL-1 on their cell surface (Fig. 4D). It will be important to determine whether the different expression patterns of DCAL-1 by DCs subsets reflect differences in the ability of these cells to capture or present Ag.

The expression of DCAL-1 by immature DCs may suggest that it is associated with Ag uptake. The possibility that DCAL-1 acts as a pattern recognition receptor of the innate immune response and can recognize particular pathogens will be the subject of future studies. However, the retention of DCAL-1 expression upon DC maturation suggests that DCAL-1 may play a role in the interaction of DCs with T cells and the cells of the adaptive immune response.

Here we demonstrate that DCAL-1 binds to CD4+ CD45RA+ T cells (Fig. 5B). This DCAL-1/T cell interaction was not inhibited by incubation with EGTA or various carbohydrates, including mannan, mannose, and fucose (data not shown). His-tagged recombinant protein containing the extracellular domains of dectin-1 also showed marked and specific binding to the surface of T cells and promoted T cell proliferation in the presence of suboptimal concentrations of CD3 mAb (10). These results suggest that dectin-1 may bind to as yet unidentified ligands on T cells, delivering T cell costimulatory signals. As DCAL-1 also bound to a population of T cells, we investigated the costimulatory potential of DCAL-1 and compared this to the costimulatory effect of DC-SIGN. Coincubation of a His-tagged DCAL-1 fusion protein with anti-CD3-stimulated T cells enhanced the proliferation and IL-4 secretion by CD4+ T cells.

The DCAL-1 fusion protein also bound to a population of CD20+ B cells in peripheral blood, but not to purified tonsillar B cells. While the significance of this is unclear, it may indicate that the expression pattern of the DCAL-1 ligand is differently regulated in B cell subsets. The precise nature of the ligand that DCAL-1 is binding on the surface of T cells and B cells remains to be defined.

In this study we have shown that coinubcation of T cells with a DCAL-1 fusion protein and anti-CD3 increases their secretion of IL-4. IL-4 plays an important role in the differentiation of both CD34+ cells and monocytes into DCs. Malignant cells may escape from the immune response in vivo because of a defective differentiation of APCs, such as DCs. Tumor cells release IL-6 and M-CSF; these cytokines inhibit the differentiation of CD34+ cells into DCs and promote their differentiation into the monocytic lineage with poorer APC capacity (41). However, IL-4 reverses the blockade of DC differentiation induced by tumor cells by reducing the expression of M-CSF and the IL-6R transducing chain (gp130), preventing the loss of GM-CSF receptor α-chain expression on

![FIGURE 8. DCAL-1 enhances the stimulatory capacity of DCs in an allogenic MLR. CD4+ CD45RA+ T cells were cocultured with different concentrations of irradiated monocyte-derived DCs in the absence or the presence of 10 μg/ml of DCAL-1 or DC-SIGN fusion proteins for 3 days. T cell proliferation was assessed on day 4 by [3H]thymidine incorporation. Results are expressed as the mean (±SD) counts per minute × 10⁶ of triplicate cultures. * and ***, p <0.05 and p < 0.001, respectively vs the control cultures with the equivalent number of stimulator cells (by Student’s t test). Results are representative of three experiments with cells isolated from different donors.](image-url)
differentiating CD34+ cells (42). The acquisition of DC-SIGN expression in the monocyte-DC differentiation pathway is primarily induced by IL-4, and GM-CSF cooperates with IL-4 to generate a high level of DC-SIGN mRNA and cell surface expression (43). The DC-associated C-type lectin, Dec-205 (CD205), is recognized by mAb MR6 (44). MR6 can also interact with the IL-4R and has an adjuvant effect in vivo (45). Although DCAL-1 is not closely related to CD205 (46), our results provide another example of C-type lectins modulating the immune response via IL-4 (Fig. 7, A and B).

Geijtenbeek et al. (34) demonstrated that the interaction between DC-SIGN and CD50 established during the initial contact between the DCs and resting T cells may contribute to the potency with which DCs can modulate T cells. Internalization motifs in the cytoplasmic tail of DC-SIGN hint at a function of DC-SIGN as an endocytic receptor. In a recent study Engering et al. (47) demonstrated that on DCs, DC-SIGN is rapidly internalized upon binding of soluble ligand. Mutating a putative internalization motif in the cytoplasmic tail reduced ligand-induced internalization. Moreover, ligands internalized by DC-SIGN are efficiently processed and presented to CD4+ T cells. However, in this study using a recombinant His-tagged DC-SIGN fusion protein we showed that the DC-SIGN/T cell interaction does not enhance CD4+ T cell proliferation and cytokine secretion (Figs. 6 and 7A).

In summary, we have identified a novel C-type lectin-like molecule, DCAL-1, that has restricted hemopoietic expression and acts as a T cell costimulatory molecule. Together with the recent reports of C-type lectins with relatively DC-restricted expression (5–15), it is becoming increasingly evident that the C-type lectin family plays a crucial role in orchestrating pathogen recognition and Ag uptake by immature DCs, while also mediating the ability of mature DCs to instruct and skew the adaptive immune response.

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References


