Dendritic Cell Immunoactivating Receptor, a Novel C-type Lectin Immunoreceptor, Acts as an Activating Receptor through Association with Fc Receptor γ Chain*

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An increasing number of C-type lectin receptors are being discovered on dendritic cells, but their signaling abilities and underlying mechanisms require further definition. Among these, dendritic cell immunoreceptor (DCIR) induces negative signals through an inhibitory immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic tail. Here we identify a novel C-type lectin receptor, dendritic cell immunoactivating receptor (DCAR), whose extracellular lectin domain is highly homologous to that of DCIR. DCAR is expressed similarly in tissues to DCIR, but its short cytoplasmic portion lacks signaling motifs like ITIM. However, a positively charged arginine residue is present in the transmembrane region of the DCAR, which may explain its association with Fc receptor γ chain and its stable expression on the cell surface. Furthermore, cross-linking of DCAR in the presence of γ chain activates calcium mobilization and tyrosine phosphorylation of cellular proteins. These signals are mediated by the immunoreceptor tyrosine-based activating motif (ITAM) of the γ chain. Thus, DCAR is closely related to DCIR, but it introduces activating signals into antigen-presenting cells through its physical and functional association with ITAM-bearing γ chain. The identification of this activating immunoreceptor provides an example of signaling via a dendritic cell-expressed C-type lectin receptor.

Paired immunoreceptors (1–4) are composed of an immunoreceptor tyrosine-based inhibitory motif (ITIM)§-bearing inhibitory receptor and an immunoreceptor tyrosine-based activating motif (ITAM)-bearing activating receptor. Such pairs of receptors, when expressed on the same cell, allow for a balance between positive and negative cell signaling. In most cases, activating receptors are formed as complexes of a ligand-binding subunit with a short cytoplasmic tail and an ITAM-bearing signal-transducing subunit. This signal-transducing subunit can be shared by several receptors. These activating receptors were first identified on effector cells such as natural killer (NK), T, B, and mast cells and subsequently detected on myeloid cells including granulocytes, macrophages, and dendritic cells (DC). Now it is evident that such receptors are used rather ubiquitously, including in platelets (5) and even nonhematopoietic cells (6). Based on their domain structures, these receptors are divided into two subgroups, Ig superfamily and Cα2+-dependent (C-type) lectin family. Besides their structural similarity, the genes for these families are clustered (e.g. the Ig superfamily in the leukocyte receptor complex on human chromosome 19 or syntenic mouse chromosome 7, and the C-type lectin family in the NK gene complex (NKC) on human chromosome 12 or syntenic mouse chromosome 6, respectively) (7).

On the other hand, there have been many C-type lectin receptors detected on DC. Based on their molecular structures, they include two types of receptors: type I C-type lectin as a type I transmembrane protein with several carbohydrate recognition domains (CRDs) and type II C-type lectin as a type II transmembrane protein with a single CRD. Both types are considered to function mainly as pattern recognition receptors for antigen capture (8) and additionally in interactions of DC with other cells (9). For example, macrophage mannose receptor and DC-SIGN may act in DC trafficking (10, 11), and DC-SIGN and DC-associated C-type lectin (Dectin)-1 can mediate an interaction of DC and T cells (12, 13). Since some of the genes for type II lectin receptors on DC are mapped close to the NKC, one might suspect their additional functions in cellular signaling.

We have previously shown that one of these receptors, DC immunoreceptor (DCIR), originally identified as an ITIM-bearing type II lectin immunoreceptor expressed on antigen-presenting cells (14), actually acts as an ITIM-dependent inhibitory receptor in B cells (15). Here we describe the further identification of DC immunoactivating receptor (DCAR) as a novel immunoreceptor closely related with DCIR, and we successfully show its activating capacity through the ITAM of its associated Fc receptor (FcR) γ chain. Our findings illustrate that DC can express potentially paired signaling immunoreceptors with C-type lectin external domains.
DCAR, a Novel Activating C-type Lectin Immunoreceptor

EXPERIMENTAL PROCEDURES

Cloning of Full-length Mouse DCAR cDNA—Total RNA, which was isolated from ICRL mouse spleen using TRIzol reagent (Invitrogen), was reverse transcribed using oligo(dT)12-18 primer and the SuperScript First-Strand Synthesis System (Invitrogen). Using this cDNA as a template, PCR was performed. TaKaRa TaqTM (Takara Shuzo Co. Ltd.) PCR System 9700 (Applied Biosystems) were as follows: 3-min denaturation at 94°C, and 5 cycles of 94°C for 4 min, 5 cycles of 94°C for 5 s and 72°C for 5 s, 5 cycles of 94°C for 5 s and 72°C for 4 min, 5 cycles of 94°C for 5 s, 70°C for 4 min, and 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s for the first or nested PCR, respectively. Rapid amplifications of cDNA ends (RACE) were performed using Marathon-Ready™ cDNA of BALB/c mouse spleen and Advantage™ 2 Polymerase Mix (Clontech) as follows: 2-min denaturation at 95°C and 40 or 30 cycles of 94°C for 10 s, 55°C for 30 s, and 72°C for 60 s for the first or nested PCR, respectively. The following primers were used: 5'-GGA CTG TCT GCC TGT TGG AAA G-3' (forward primer), 5'-AGT CCC TGA AAT GTA TTT TCT TCT TCA TCT G-3' (reverse primer). Conditions adapted for Gene-Cloning PCR System 9700 (Applied Biosystems) were as follows: 5-min denaturation at 95°C, 40 cycles of 94°C for 5 s, 50°C for 5 s, and 72°C for 30 s. The amplified cDNA fragments were purified and subcloned into pGEM®-T Easy Vector (Promega), and their base sequences were determined. The DCAR-FcR chimera containing the cytoplasmic to transmembrane portion of DCAR (nucleotides 101–244) attached to C-terminal FLAG-tagged extracellular Fc γ receptor IIB (nucleotides 94–612 of Gen-Bank™, U51629) (24) was created by PCR and subcloned into pAPuro vector (25). Untagged γ chain and mutated γ chain were subcloned into pAneo vector (26). After confirmation of their base sequences, the DCAR-FcR construct was introduced with or without the γ chain construct into A20. The DCAR-FcR chimera was electroporated into A20.6.16 cells by electroporation and selected in the presence of puromycin (8 μg/ml) with or without G418 (2 mg/ml), respectively. pAPuro and pAneo vectors were kindly provided by Dr. Tomohiro Kuroasaki (Kansai Medical University, Moriguchi, Japan). Cell surface expression levels of FLAG-tagged DCAR and DCAR-FcR were analyzed with flow cytometry (FCM) using anti-FLAG M2 monoclonal Ab (Sigma) and phycocerythrin-conjugated anti-CD16/CD32 monoclonal Ab 2.4G2 (BD Pharmingen), respectively. FCM analysis was performed using FACSanalysis™ and CELLQuest™ software (BD Immunocytometry Systems). The expression level of γ chain was analyzed with immunoprecipitation and Western blotting using anti-γ polyclonal Abs (kindly provided by Dr. Hiroshi Takayama, Kyoto University, Kyoto, Japan). Fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (Jackson Immunoresearch Laboratories) and horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham Biosciences) were used as the second Abs. For formation of immune complexes (IC), NALE™ 2.4G2 (BD Pharmingen) and mouse anti-rat IgG F(ab’)_2 fragments (Jackson Immunoresearch Laboratories) were incubated on ice for more than 30 min (27). The F(ab’)_2 fragment of rabbit anti-mouse IgG (H + L) (Zymed Laboratories Inc.) and horseradish peroxidase-conjugated anti-phosphotyrosine monoclonal Ab PY20 (BD Pharmingen) were also purchased. 

RT-PCR Analysis—Oligo(dT)12-18 primed cDNAs of mouse tissues, BM-DC, and BM-NK cells were prepared and provided for PCR under the same conditions as described above (30 cycles for DCAR and DCIR and 25 cycles for glyceraldehyde-3-phosphate dehydrogenase). The following primer pairs were used for DCAR, 5'-GAG TTC TGG CCT TGT TGG TAA AAG-3' (forward primer) and 5'-GAC ACG AGC TTC AGA CTT TG-3' (reverse primer); for DCIR, 5'-GAT CTA AAG GCT GGT GAG GAG G-3' (forward primer) and 5'-GAT CTA AAG GCT GGT GAG GAG G-3' (reverse primer); for glyceraldehyde-3-phosphate dehydrogenase, 5'-AAT GGT GAA GGT CAG TGT GAA CG-3' (forward primer) and 5'-TCT TGC TCA GTG TCG TCC TG-3' (reverse primer).

Immunoprecipitation and Western Blotting—To detect association of DCAR and γ chain, transfected 293T cells were solubilized in 1% digitonin/mimixed buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1 mM Na_3VO_4, 0.5% Triton X-100, 10 μg/ml each of leupeptin, aprotinin, and pepstatin A, 1 mM PMSF, and 5 mM 2-μg/ml each of leupeptin, aprotinin, and pepstatin A, at 0°C for 30 min. The cell lysates were centrifuged at 10,000 × g for 30 min. The supernatants (postnuclear supernatants) were incubated on ice with immunoprecipitation and Western blotting using anti-DCAR and anti-FLAG monoclonal Abs (kindly provided by Dr. Hiroshi Takayama, Kyoto University, Kyoto, Japan). Fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (Jackson Immunoresearch Laboratories) and horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham Biosciences) were used as the second Abs. For formation of immune complexes (IC), NALE™ 2.4G2 (BD Pharmingen) and mouse anti-rat IgG F(ab’)_2 fragments (Jackson Immunoresearch Laboratories) were incubated on ice for more than 30 min (27). The F(ab’)_2 fragment of rabbit anti-mouse IgG (H + L) (Zymed Laboratories Inc.) and horseradish peroxidase-conjugated anti-phosphotyrosine monoclonal Ab PY20 (BD Pharmingen) were also purchased.

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Immunoprecipitation and Western Blotting—To detect association of DCAR and γ chain, transfected 293T cells were solubilized in 1% digi-
tonin buffer (1% digitonin, 0.12% Triton X-100, 150 mM NaCl, and 20 mM triethanolamine, pH 7.8) (28). Precleared cell lysates were incubated with preformed recombinant Protein A-Sepharose (Zymed Laboratories Inc. conjugated with anti-FLAG M2. To see expression of intact or mutant \( \gamma H9253 \) chain, transfected 293T or A20 IIA1.6 cells were solubilized in 1% digitonin buffer, and the precleared lysates were incubated with anti-\( \gamma H9253 \)-conjugated recombinant Protein A-Sepharose. For detection of protein tyrosine phosphorylation, A20 IIA1.6 transformants (1.5 \( \times 10^7 \) cells/ml) were stimulated with IC (mixture of 2.5 g/ml 2.4G2 and 75 g/ml mouse anti-rat IgG F(ab')2) or 25 g/ml F(ab')2 anti-mouse IgG at 37 °C for 1 min until the addition of ice-cold phosphate-buffered saline and sequentially solubilized in 1% Nonidet P-40 buffer (1% Nonidet P-40, 20 mM Tris, 100 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM sodium vanadate, pH 7.4) (29). Precleared lysates were incubated with agarose-conjugated PY20 (BD Pharmingen). Complete protease inhibitor mixtures (Roche Applied Science) were added to both lysis buffers. Immunoprecipitates were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with appropriate Abs. Immuno blotted proteins were visualized by chemiluminescence using Western Lightning™ Chemiluminescence Reagent Plus (PerkinElmer Life Sciences).

Calcium Mobilization Assay—A20 IIA1.6 transformants (8 \( \times 10^6 \) cells/ml) were loaded with 10 \( \mu \)M Fura Red (Molecular Probes, Inc., Eugene, OR) in 10 mM Hepes-containing medium at 37 °C for 30 min, inverted frequently (15). Collected cells were resuspended in the same medium (1.6 \( \times 10^6 \) cells/ml). After the addition of IC (mixture of 1 g/ml 2.4G2 and 30 g/ml mouse anti-rat IgG F(ab')2 or 10 g/ml F(ab')2 anti-mouse IgG, the cells were analyzed with FCM.

RESULTS

Identification of the cDNA of DCAR—Using a search of cDNA and amino acid sequences homologous to the mouse DCIR, one uncharacterized but highly homologous gene was found. This gene, 1810046I24Rik, was originally obtained from the full-length cDNA library of mouse pancreas and appears in GenBank™ under the accession number NM_027218 (30). To confirm the nucleotide sequence of its predicted coding region,
heminested RT-PCR was performed using spleen mRNA as a template. The obtained products were separated in agarose gel electrophoresis, and one major band and minor bands with various sizes were observed. DNA was extracted from the major band to determine its base sequence, and two isoforms were identified as the result. The major isoform was 99 base pairs longer than the other minor one, identical to NM_027218, and these were named DCAR α and β, respectively. To isolate the 5′- and 3′-untranslated regions of DCAR, RACE experiments were performed, and the base sequences of the obtained cDNA fragments were determined. A contiguous contig representing the full-length cDNA of DCAR was composed by alignment of these three sequences including results of 5′-RACE, RT-PCR, and 3′-RACE, each of which represented at least six different clones. This contig is 737 base pairs in length, excluding the poly(A) tail, and contains a putative open reading frame of 627 base pairs (Fig. 1A). Given a potential start codon is present in the consensus Kozak sequence (31) and a stop codon exists at an upstream position (TAG: nucleotides 89–91), the encoded protein should contain 209 amino acids. The presence of a putative hydrophobic signal anchor, consisting of 27 amino acids (residues 15–41; underlined in Fig. 1A), indicates that this polypeptide is a type II transmembrane protein. Since its extracellular domain contains a single CRD, DCAR represents a type II C-type lectin.

Genomic Structure and Localization of the DCAR Gene—The 1810046I24Rik gene, identical to DCARβ, is localized on mouse chromosome 6 supercontig Mm6_WIFebo1_114 (GenBankTM accession number NW_000264). Nucleotide sequence alignment of DCAR and this supercontig enabled us to demonstrate the genomic structure of the DCAR gene with appropriate exon-intron boundaries except for exon III, which was deleted in the β isoform. The DNA fragments containing introns II and III were obtained by PCR, and their exon-intron boundaries were confirmed. The whole structure of the DCAR gene is shown in Fig. 1B. It is composed of six exons representing functional domains. Exon I encodes the 5′-untranslated region and 8 amino acids in the cytoplasmic domain, exon II encodes 30 amino acids mainly containing the transmembrane domain (TM in Fig. 1), exon III encodes 33 amino acids representing the neck domain, and the remaining CRD is encoded by 3 exons (IV, V, and VI). DCAR maps close to DCIR, and their genes form a cluster with other related C-type lectins including Dectin-2, macrophage-restricted C-type lectin (MCL) (32), and macrophage-inducible C-type lectin (MinCle) (33) in a short region of the NW_000264 supercontig (Fig. 1C). The human genomic locus syntenic to this cluster is reportedly located on chromosome 6 supercontig Mm6_WIFeb01_114 (GenBankTM accession number NW_000264). Nucleotide sequence alignment of DCAR and this supercontig enabled us to demonstrate that they contain quite similar numbers of amino acids and considerable sequence homology (Fig. 2B), especially the cytoplasmic to neck portions (82% identity for DCAR and Dectin-2). Notably, the charged arginine (DCAR and Dectin-2) and lysine (hBDCA-2) residues were present at the conserved position in their transmembrane domains, and these have been reported to be responsible for an association with adaptor molecules in many immunoreceptors.

Expression Profile of DCAR Compared with That of DCIR—Specific expression of DCAR was only examined by RT-PCR, because it was considered to be quite difficult to produce specific Ab recognizing the extracellular domain of DCAR, due to the high sequence homology between the CRDs of DCAR and DCIR as well as the neck domains of DCAR and Dectin-2. The useful forward primer specific for DCAR was prepared in its 5′-untranslated region. Among normal tissues, DCAR transcripts were detected strongly in lung and spleen and weakly in skin and lymph node, whereas DCIR transcripts were more ubiquitously observed (Fig. 3). During differentiation of BM-DC, DCAR expression reached a maximum at day 8 versus day 10 in the case of DCIR. BM-NK cells expressed neither DCAR nor DCIR. These data indicate that the expression pattern of DCAR is similar to but not identical to DCIR.

Association of DCAR with Fcγ Chain—When transfected in 293T cells, FLAG-tagged DCAR by itself was expressed very weakly on the cell surface, in contrast to strong expression of DCIR and almost no expression of Dectin-2 on the transfec-tants (Fig. 4A, top column). To determine whether an adaptor molecule can associate with DCAR, 293T cells were transfected with a mixture of FLAG-tagged DCAR and each one of the known adaptor molecules, including DAP12, DAP10, CD3 ζ chain, and FcR γ chain, and analyzed with FCM. Only in the case of cotransfection with γ chain was surface expression of DCAR significantly enhanced (Fig. 4A, middle two columns). Transfection of γ chain alone could not induce any protein expression on the cell surface detected by anti-FLAG M2 (data not shown). The association of DCAR and γ chain was further confirmed by immunoprecipitation. 293T cells transfected with FLAG-tagged DCAR and γ chain were lysed, and the lysates were immunoprecipitated with anti-FLAG M2. The immunoprecipitates were separated by SDS-PAGE, and the associated γ chain was visualized with immunoblotting (Fig. 4B). These
data indicate that DCAR and γ chain interact in the cotransfected 293T cells and that this interaction is involved in transport of DCAR to the cell surface. Furthermore, to examine the contribution of membranous arginine residue of DCAR to this association, two kinds of mutant DCAR, whose arginine was mutated to noncharged isoleucine (R/I) and negatively charged aspartic acid (R/D), were prepared and used for cotransfection experiments. Unexpectedly, R/I and R/D mutations only partially but similarly reduced the cell surface expression of DCAR in the presence of γ chain (bottom column of Fig. 4A), indicating that the conserved charged arginine residue in the transmembrane domain of DCAR is at most partly responsible for the association with γ chain. Mutation of ITAM-tyrosines in the γ chain (amino acids 65 and 76 mutated to phenylalanines; Y/F) did not influence the expression level of DCAR cotransfected with intact γ chain (bottom column of Fig. 4A). This result indicates that the ITAM of γ chain has no effect in this association.

Calcium Mobilization after Ligation of DCAR-FcR through the ITAM of Associated γ Chain—An Fc γ receptor-negative derivative of mouse A20 B cells, IIA1.6, which we previously used for detection of inhibitory function of DCIR (15), did not influence the expression level of DCAR as well as the role of γ chain in DCAR-mediated activation. For this purpose, a chimeric receptor containing the cytoplasmic to transmembrane portion of DCAR

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**Fig. 4. Association of DCAR with FcR γ chain in 293T cells.** A, 293T cells were transiently transfected with FLAG-tagged DCAR or related lectins (top column), with a mixture of FLAG-tagged DCAR and ITAM-containing adaptor molecules (middle two columns), and with a mixture of FLAG-tagged mutant/intact DCAR and intact/mutant γ chain (bottom column), labeled with anti-FLAG, and analyzed with FCM. Broken lines represent FCM results of parental 293T cells (top column), those of the cells expressing DCAR alone (middle two columns), and those of the cells expressing DCAR with γ chain (bottom column). B, lysates of 293T transformants were immunoprecipitated with anti-FLAG, and, after separation with 15% SDS-PAGE, the precipitates were probed with anti-γ (upper panel). The same series of lysates were also immunoprecipitated with anti-γ, and the precipitates were probed with the same Ab (lower panel).
and extracellular domain of mouse Fc γ receptor IIB (DCAR-FcR) was constructed and transfected into A20 IIA1.6 cells. After analyzing the expression level of DCAR-FcR on the surface of the transformants with FCM, one clone clearly expressing DCAR-FcR was selected. After sequential transfection of the transformants with FCM, one clone clearly expressing DCAR-FcR plus γ chain, and DCAR-FcR plus mutant γ chain, respectively. B, transformed A20 IIA1.6 cells were lysed and immunoprecipitated with anti-γ. The immunoprecipitates were separated in 15% SDS-PAGE gel and probed with the same Ab.

FIG. 5. Expression of DCAR-FcR and Fcγ γ chain in A20 IIA1.6 transformants. A, A20 IIA1.6 transformants were labeled with phycoerythrin-conjugated 2.4G2 and analyzed with FCM. Finely broken, solid, and roughly broken lines represent the FCM result of transformant with DCAR-FcR alone, DCAR-FcR plus γ chain, and DCAR-FcR plus mutant γ chain, respectively. B, transformed A20 IIA1.6 cells were lysed and immunoprecipitated with anti-γ. The immunoprecipitates were separated in 15% SDS-PAGE gel and probed with the same Ab.

We first examined the effect of DCAR-FcR ligation on Ca²⁺ mobilization after ligation of DCAR-FcR. A20 IIA1.6 transformants shown in Fig. 5 were loaded with Fura Red and analyzed with FCM after stimulation with F(ab’)2 anti-mouse IgG (lower column) or IC made of 2.4G2 and mouse anti-rat IgG F(ab’)2 (upper column). The arrowheads indicate the time points of adding stimulators. B, tyrosine phosphorylation of cellular proteins after ligation of DCAR-FcR. After stimulation of the A20 IIA1.6 transformant expressing DCAR-FcR plus γ chain with various stimuli (left panel) or stimulation of various transformants with IC (right panel), the cells were lysed, immunoprecipitated with anti-Tyr(P), separated in 9% SDS-PAGE gel, and probed with the same Ab.

FIG. 6. The complex of DCAR-FcR and Fcγ γ chain transmits activation signals depending on the ITAM of γ chain. A, calcium mobilization after ligation of DCAR-FcR. A20 IIA1.6 transformants shown in Fig. 5 were loaded with Fura Red and analyzed with FCM after stimulation with F(ab’)2 anti-mouse IgG (lower column) or IC made of 2.4G2 and mouse anti-rat IgG F(ab’)2 (upper column). The arrowheads indicate the time points of adding stimulators. B, tyrosine phosphorylation of cellular proteins after ligation of DCAR-FcR. After stimulation of the A20 IIA1.6 transformant expressing DCAR-FcR plus γ chain with various stimuli (left panel) or stimulation of various transformants with IC (right panel), the cells were lysed, immunoprecipitated with anti-Tyr(P), separated in 9% SDS-PAGE gel, and probed with the same Ab.

We report the identification and characterization of a novel C-type lectin immunoreceptor, DCAR. Similar to other paired immunoreceptors, DCAR and DCIR share highly homologous extracellular domains, whereas their intracellular domains are quite different; the DCAR domain is very short and lacks signaling motifs, whereas that of DCIR is longer and contains an ITIM. The presence of a charged arginine residue in the transmembrane domain of DCAR encouraged us to investigate the possibility that DCAR acts as an activating receptor in association with an adaptor molecule. The most widely used ITAM-
bearing adaptor molecule would be DAP12 (38), and its critical role in DC antigen-priming capacity as well as in NK cell activation was demonstrated by the analysis of DAP12-deficient mice (39, 40). In fact, myeloid DAP12-associating lectin-1 (41), triggering receptor expressed on myeloid cells-2 (42), and signal-regulatory protein b (43, 44) have been identified as DAP12-associating immunoreceptors expressed in myeloid cells. In contrast, Ig-like transcript-1 (45) and paired Ig-like receptor-A (28) are associated with FcRγ chain. Our results clearly show that DCAR is an additional receptor physically and functionally associating with γ chain. Tomasello et al. (44) compared the positions of charged amino acid residues in the transmembrane domains of DAP12-associating receptors with those of γ chain-associating receptors and showed their distinctive structural features: the former in the center of the transmembrane domain and the latter close to the extracellular domain. It should be noted that a position near the ectodomain in a type I membrane protein corresponds to a position close to the cytoplasm in the case of type II proteins, such as NK-R1A.

Consistent with this role, the position of the membranous arginine residue of DCAR, which associates with γ chain, is very close to its intracellular domain. Since a previous study showed a critical role for the negatively charged aspartic acid of DAP12 in its association with myeloid DAP12-associating lectin-1 (41), we examined the importance of the positively charged arginine of DCAR. Unexpectedly, mutation analyses could not demonstrate an essential role of this charged amino acid for an interaction with γ chain, only a partial contribution.

Dectin-2 and hBDCA-2 also have a charged arginine and lysine residue, respectively, in the conserved position of their transmembrane domains, suggesting that these two receptors also work as activating receptors in the presence of appropriate adaptor molecules. However, cotransfection with any of the four adaptor molecules (DAP12, DAP10, CD3ζ, and FcRγ) could not enhance the surface expression of Dectin-2 at all (data not shown). As Fernandes et al. (35) proposed, Dectin-2 (NKCL) might associate with an as yet unidentified signaling molecule. Another possibility might be that Dectin-2 with no signaling capacity can form a heterodimer with DCAR and/or DCIR, similar to the CD94 NK cell receptors, which form heterodimers with activating NKG2C and inhibitory NKG2A molecules (46). In contrast, triggering hBDCA-2 can induce Ca2+ mobilization and protein tyrosine phosphorylation, suggesting that these activation signals are mediated by some associated adaptor molecule (36). The FcRγ chain would be the most possible candidate, considering its sequence similarity with DCAR. Although it has been proposed that Dectin-2 represents the mouse homologue of hBDCA-2 (36), we have newly identified the predicted human counterpart of Dectin-2, which is clearly distinct from hBDCA-2,2 and it is possible that Dectin-2 and hBDCA-2 have distinctive roles.

We have successfully shown the in vitro activatory effects of cross-linking chimeric DCAR-FcR and its associated FcRγ chain, as in Ca2+ mobilization and protein tyrosine phosphorylation. The association with ITAM-bearing γ chain is critical for the signal transduction we observed. Compared with the inhibitory effects of DCIR shown by the similar experiments (15), the function of DCAR shows a clear contrast with DCIR, further suggesting that they form a functional pair. Unfortunately, their natural ligands are unknown, and their in vivo functions remain to be resolved. Similar to many paired immunoreceptors, their extracellular CRDs are highly homologous but are not completely the same. This fact raises two possibilities: the same ligand can bind both receptors with different affinity, or similar but distinct molecules bind to the specific receptors. Indeed, the EPS sequence is conserved in both receptors as well as the human counterpart of DCIR, but specific carbohydrates that bind to the CRD containing this motif have not yet been identified. Human BDCA-2, which has activating properties in vitro, further inhibits interferon-α/β production by plasmacytoid DC (36). Engering et al. (47) have proposed an immune escape theory in which virus binding to hBDCA-2 can down-regulate plasmacytoid DC function by inhibiting activation signals mediated by pathogen recognition receptors such as Toll-like receptors. Both DCAR and DCIR can also possibly modulate intracellular signaling induced when pathogens or self-antigens act through their specific receptors.

Our RT-PCR analyses showed that the specific transcripts of these receptors had a similar but clearly distinct pattern. The observed ubiquitous expression of inhibitory however, DCIR in a variety of tissues may possibly contribute to the maintenance of the immunological homeostasis. It should be noted that DCAR mRNA expression by itself does not represent its surface protein expression, because DCAR can sufficiently be expressed on the cell surface only in the presence of FcRγ chain. Considering that the expression level of γ chain does not remarkably change during maturation of BM-DC (data not shown), however, DCAR mRNA expression may control its surface protein expression in these cells. On day 8 and day 10, mRNA expression of DCAR and DCIR reached maximum, respectively, possibly contributing to the observation that T cell activating capacity reached a maximum on day 8, whereas the number of apoptotic cells increased on day 10 (16). Although expression of DCAR and DCIR was not specifically observed in DC, their expression in each DC subset or regulation of their expression should be examined to further determine their roles in the DC system.

In our previous report, rabbit polyclonal Abs raised against the two polypeptides in the CRD of DCIR were used for detecting DCIR protein in vivo (15). Amino acid sequence alignment revealed that one polypeptide (GHRQWQWVDQTPYEES) was fully identical to the corresponding region of DCAR and that the other (QSQEEQDFITGILDTH) was not the same but was quite similar to the corresponding DCAR polypeptide (14 of 16 residues identical). Thus, the published data of the FCM analysis using the Ab for the former polypeptide (15) should be reinterpreted to indicate that either DCIR or DCAR is expressed on the surface of major histocompatibility complex class II-positive antigen-presenting cells. Although FCM analysis with both Abs revealed almost the same expression pattern (15), suggesting the expression of DCIR and DCAR on the same cell surface, precise expression profiles for each molecule need to be determined at the single cell level. For this reason, we cannot definitely conclude that DCIR and DCAR are paired immunoreceptors. Preparation of monoclonal Ab recognizing each molecule would resolve this issue.

In conclusion, we have reported the molecular cloning and functional characterization of DCAR, a novel C-type lectin immunoreceptor expressed on DC. DCAR mediates activating signals through associated FcRγ chain and represents a potent immunoreceptor forming a pair with inhibitory DCIR. The presence of such putative paired immunoreceptors among DC-expressing C-type lectins provides a possible model of their signaling capacity.

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