APCs Express DCIR, a Novel C-Type Lectin Surface Receptor Containing an Immunoreceptor Tyrosine-Based Inhibitory Motif


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We have identified a novel member of the calcium-dependent (C-type) lectin family. This molecule, designated DCIR (for dendritic cell (DC) immunoreceptor), is a type II membrane glycoprotein of 237 aa with a single carbohydrate recognition domain (CRD), closest in homology to those of the macrophage lectin and hepatic asialoglycoprotein receptors. The intracellular domain of DCIR contains a consensus immunoreceptor tyrosine-based inhibitory motif. A mouse cDNA, encoding a homologous protein has been identified. Northern blot analysis showed DCIR mRNA to be predominantly transcribed in hematopoietic tissues. The gene encoding human DCIR was localized to chromosome 12p13, in a region close to the NK gene complex. Unlike members of this complex, DCIR displays a typical lectin CRD rather than an NK cell type extracellular domain, and was expressed on DC, monocytes, macrophages, B lymphocytes, and granulocytes, but not detected on NK and T cells. DCIR was strongly expressed by DC derived from blood monocytes cultured with GM-CSF and IL-4. DCIR was mostly expressed by monocyte-related rather than Langerhans cell related DC obtained from CD34+ progenitor cells. Finally, DCIR expression was down-regulated by signals inducing DC maturation such as CD40 ligand, LPS, or TNF-α. Thus, DCIR is differentially expressed on DC depending on their origin and stage of maturation/activation. DCIR represents a novel surface molecule expressed by Ag presenting cells, and of potential importance in regulation of DC function.


APCs Express DCIR, a Novel C-Type Lectin Surface Receptor Containing an Immunoreceptor Tyrosine-Based Inhibitory Motif

Dendritic cells (DC) represent a highly specialized type of APC that constitute a leukocyte population playing a sentinel function by their ability to capture Ag. Following Ag capture, DC migrate to secondary lymphoid organs, where they display a unique capacity to present processed Ag and activate naive T cells, thus initiating a specific primary T cell response (reviewed in Refs. 1 and 2).

The functions of DC are mediated in part by the expression of specialized surface receptors (3). In the case of Ag uptake and presentation, DC express the macrophage-mannose receptor (MMR) (4–6) and DEC-205 (7) that bind and internalize carbohydrate-bearing Ags by receptor-mediated endocytosis. Both MMR and DEC-205 are type I membrane proteins that belong to the Ca2+-dependent (C-type) lectin family (8). Like the pancreatic phospholipase A2 receptor (9, 10), they possess multiple Ca2+-dependent extracellular carbohydrate recognition domains (CRDs) on their NH2 terminus. This group of type I surface lectins also includes members of the selectin family that are implicated in adhesion events on endothelial cells, which mediate the tethering and rolling of leukocytes via oligosaccharide groups (11).

A second family of C-type lectin molecules are type II proteins with a single CRD at the COOH terminal end. This group includes cell surface receptors such as the hepatic asialoglycoprotein receptors (ASGPRs) 1 and 2 (12, 13) and the macrophage lectin (14), which bind oligosaccharide groups and are involved in ligand internalization and may thus be important in the uptake of Ag by macrophages.

The receptors coded for by genes localized on human chromosome 12p12-p13 in a region designated as the NK gene complex (15, 16) represent a closely related group of type II proteins, although their extracellular C-terminal domains are relatively divergent from the CRDs in the C-type lectins (17). This group of molecules includes products of the NKG2 gene complex (18), CD94 (19), NKR-P1A/CD161 (20), and CD69 (21–23). Some of these receptors are involved in recognition of MHC class I molecules and in the regulation of NK cell activity. In particular, inhibition of cellular functions by NKG2A/B/CD94 heterodimers is linked to the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the NKG2A/B intracellular domains (24). The ITIM, first identified in FcγRIIB (25–27), is also present in the cytoplasmic tail of NK inhibitory receptors of the Ig-like superfamily (28–31).

To identify novel receptors of DC that may have important functions in Ag-presenting cells, we searched nucleotide databases with a sequence (8 aa) derived from the CRD of hepatic ASGPRs and the macrophage lectin. In the present report, we describe the identification of a cDNA encoding DCIR (for DC immunoreceptor), a novel type II...
molecule of the C-type lectin family containing an intracellular ITIM consensus sequence.

Materials and Methods

Hematopoietic factors, cytokines and reagents

All cultures were performed in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine (all from Life Technologies, Gaithersburg, MD), and 160 μg/ml gentamicin (Schering-Plough, Levallois-Perret, France). All hematopoietic factors and cytokines are recombinant human proteins. GM-CSF (sp. act.: 2 × 10^7 U/mg; Schering-Plough Research Institute, North, NJ) was used at a concentration of 100 ng/ml (200 U/ml). TNF-α (sp. act.: 2 × 10^7 U/mg; Genzyme, Boston, MA) was used at an optimal concentration of 2.5 ng/ml (50 U/ml). Stem cell factor (SCF; sp. act. 4 × 10^7 U/mg; R&D Systems, Abington, U.K.) and M-CSF (sp. act.: 2 × 10^8 U/mg; R&D Systems) were used at an optimal concentration of 25 ng/ml. GM-CSF (ESO, 0.01–0.03 ng/ml; R&D Systems) was used at an optimal concentration of 25 ng/ml. IL-4 (sp. act.: 10^9 U/mg; Schering-Plough Research Institute) was used at 50 U/ml. In some experiments, cells were activated with 1 ng/ml PMA (Sigma, St. Louis, MO) and 1 μg/ml ionomycin (Calbiochem, La Jolla, CA) or with 25 ng/ml LPS (Sigma).

Cell preparations

Umbilical cord blood samples, peripheral blood samples, and tonsils were obtained according to institutional guidelines. PBMC were purified from human peripheral blood by Ficoll-Hyphaque centrifugation. Monocytes were purified from PBMC by centrifugation over a 50% Percoll gradient followed by immunomagnetic depletion of contaminating T, B, and NK cells using a mixture of mAbs composed of OKT3 (OKT8, CD8), and 4G7 (CD19, all ascites from our laboratory), NKH-1 (CD56) (Coulter, Miami, FL), ION16 (CD14) and anti-glycophorin A (both from Immunotech, Marseille, France), and goat anti-mouse Ig beads (Dynabeads MoA; Dynal, Oslo, Norway). The isolated cells were >95% CD14+ as judged by staining with anti-CD14 mAb and flow cytometric analysis. T lymphocytes were purified from PBMC by immunomagnetic depletion using a mixture composed of mAbs IOM2 (CD14), ION16 (CD16), IOT17 (CD35), and ION2 (HLA-DR) from Immunotech, mAb 89 (CD40, produced in our laboratory) (32), NKH-1 (CD56), and 4G7 (CD19). The purity of CD3+ T cells (CD4+ and CD8+) was >95%. Granulocytes were purified from whole blood by centrifugation in Ficoll to pellet RBCs and polymorphonuclear cells. The granulocyte fraction was then purified with Polymorphrep (Nycomed, Oslo, Norway). B cells were isolated from tonsils essentially as previously described (33). Briefly, T lymphocytes were first depleted by rosetting with SRBCs. The residual non-B cells were further depleted with T cell-specific (CD2, CD3, and CD4) and monocyte-specific (CD14) Abs, followed by immunomagnetic depletion. The resulting population was >98% CD19+ B cells. CD34+ hematopoietic progenitors were purified from umbilical cord blood as previously described (34). Briefly, cells bearing CD34 Ag were isolated from mononuclear fraction through purification from umbilical cord blood as previously described (34). Briefly, some experiments, cells were activated with PMA and ionomycin for 1 and 6 h, then pooled. Activated and nonactivated cells were lysed for RNA extraction.

Generation of DC from CD34+ progenitors and from monocytes

Cultures of CD34+ cells were established in the presence of SCF, GM-CSF, TNF-α, and 5% AB+ pooled human serum, as described (34, 35). By day 5–6, human serum was removed and cells were further cultured in the presence of GM-CSF and TNF-α until day 12. At this time point, aliquots of cells were activated with PMA and ionomycin for 1 and 6 h, then pooled and lysed for RNA extraction. For analysis of DCIR expression by flow cytometry, cells were collected at the time points indicated within the text. In some experiments CD1a+ and CD14+ DC precursor subsets were separated at day 6 by flow cytometry and further cultured until day 12 in the presence of GM-CSF, with or without TNF-α. Monocyte-derived DC were produced by culturing purified blood monocytes for 6 days in the presence of GM-CSF and IL-4 (36). In some experiments, 5 × 10^4 monocyte-derived DC/well (24-well culture plate) were further activated with LPS at the concentration of 25 ng/ml for 72 h or by coculture with 4 × 10^4 irradiated (7,500 rads) murine fibroblastic L cells transfectected with the cDNA for CD40 ligand (33).

Northern and Southern blot analyses

Human mRNA adult tissue blot was used (MTN blot 7760-1, 7759-1, and 7767-1 and Immune system (MTN) blot 7754-1; Clontech, Palo Alto, CA). Southern blots were prepared from human genomic DNA isolated from PBMC (37). Genomic DNA was cut with the enzymes BamHI or SacI under standard conditions, and fragments were separated on a 1% TAE agarose gel, then transferred to Hybond N (Amersham, Uppsala, Sweden) overnight in 0.5 M NaOH and 10× SSC. Hybridization of Southern and northern DNA blots was with a 391-bp DNA fragment from 3′ end of the DCIR cDNA, produced by PCR amplification of a region defined by the oligonucleotides 5′-GATAAAACCTCAAGAGACGAG-3′ (forward primer) and 5′-CCTAATTTCTCTAGCCCTTGTAC (reverse primer). This fragment was labeled with [32P]dCTP using the High Prime kit (Boehringer Mannheim, Meylan, France). Unincorporated nucleotides were removed by spin column chromatography (Chromaspin-100, Clontech). Membranes were prehybridized and hybridized under standard conditions (37). Low and high stringency washes were at 2× SSC/0.2% SDS and 0.2× SSC/0.2% SDS respectively, each done twice for 30 min. The membranes were incubated with Biomax MR film (Kodak, Rochester, NY) for 21 days.

RNA, DNA, and RT-PCR analysis

Cells were lysed and total RNA was extracted (38) and used for the preparation of first-strand cDNAs. First-strand cDNAs were prepared after DNase I treatment (in the presence of RNase inhibitor) of 5 μg of total RNA using oligo(dT) primers (Pharmacia, Uppsala, Sweden) and the Superscript kit. Synthesis of cDNAs was controlled by performing RT-PCR using β-actin primers. RT-PCR with the primers 5′-CTGAG GAAAGGCCTCTTGGAGACTGCG (forward primer) and 5′-CAT TCTTTGACAGTCCTTACGTC (reverse primer) specific for human DCIR (hDCIR) cDNA (1 ng/ml) was performed using the AmpliTaq enzyme and buffer (Perkin-Elmer, Paris, France), dNTPs at 0.8 mM, and DMSO at a 5% final concentration. Cycle conditions were 92°C for 1 min, 60°C for 2 min, and 72°C for 3 min for 35 cycles. PCR products were cloned using the pCRII vector (TA cloning kit, Invitrogen, San Diego, CA). Double-stranded plasmid DNA was sequenced on an ABI 373A sequencer (Applied Biosystem, Foster City, CA) using dye terminator technology. Sequencher (Code Codes, Ann Arbor, MI) and Lasergene (DNA-STAR, London, U.K.) software was used to analyze sequences. Comparisons against the GenBank databases were using the BLAST algorithm.

Chromosomal localization of hDCIR gene

Chromosomal localization was performed with the Stanford G3 RH medium resolution panel (Research Genetics, Huntsville, AL). PCR was as described above using oligonucleotides which overlapped both sides of the hDCIR gene (forward 5′-GGTCCAATCTGGTGGCCTCT GCT, reverse 5′-GGTTTAGGCCTGAGGACTT). The results were scored manually and analysis was performed with the RHMAPPER program (http://shgc-www.stanford.edu).

Production of recombinant DCIR and generation of anti-DCIR mAbs

hDCIR exodomain-IgG and HRP fusion proteins were produced for production and screening of mAbs, respectively. A XhoI fragment was derived from PCR with hDCIR cDNA as template and primers designed to amplify hDCIR residues 448–951, which encode the extracellular domain, incorporated into the XhoI site of the plasmid pcDNA3.1(+) (Invitrogen) and then cloned into the NotI and XhoI sites of the pCDM8 vector. The resulting expression vector was transfected into 293T cells, and the recombinant hDCIR protein was purified from the supernatant by Ni-NTA chromatography (Pharmacia). For production of the hDCIR-HRP fusion protein, COPS cells were transfected with the hDCIR-HRP vector using the FuGENE 6 transfection reagent (Boehringer Mannheim) according to the manufacturer’s instructions. After 4–5 days of

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culture in RPMI 1640 medium without phenol red and FCS, the cell supernatant was collected and used as source of the protein. mAbs against hDCIR were produced by immunizing BALB/c mice (Iffa Credo, Les Oncins, France) with three i.p. injections of 2 μg purified DCIR-Ig fusion protein in Freund’s adjuvant (Sigma). Spleens were removed 3 days after a final i.v. injection of DCIR-Ig, and splenocytes were fused with murine SP2 myeloma cell line using polyethylene glycol-1000 and cultured in 96-well plates using standard procedures. Hybridoma supernatants were screened for their reactivity against hDCIR-HRP fusion protein by ELISA. Briefly, supernatants were incubated in microtiter plates overnight, and plates were washed twice, 100 μl of TMB Microwell peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). After washing, 100 μl of 3-amino-ethyl carbazole (Sigma) was added to stop the reaction, and plates were read at 650 nm in an ELISA reader. Anti-DCIR mAbs were purified from the supernatant by affinity chromatography using a monoxydine-agarose matrix as described previously.

Flow cytometric analysis and cell sorting

Cell-surface expression of DCIR was determined by immunofluorescence staining and flow cytometric analysis with a FACSCalibur (Becton Dickinson, Mountain View, CA). For single staining, cells were incubated for 30 min at 4°C with 10 μg/ml purified anti-DCIR mAb 111F8, then washed twice in PBS, 1% BSA, and 0.1% NaN3, and labeled with PE-conjugated F(ab’)2 goat anti-mouse IgG (Fc region-specific; Jackson ImmunoResearch, West Grove, PA). After two washes, 100 μl of supernatants of COP5 cells transfected with the DCIR-HRP vector were added and plates were incubated for 2 h at room temperature. Plates were then washed twice, and the presence of anti-DCIR mAbs complexed to DCIR-HRP fusion protein was determined by adding temperature. Plates were then washed twice, and the presence of anti-DCIR mAbs complexed to DCIR-HRP fusion protein was determined by adding 100 μl of TMB Microwell peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). After cloning of selected hybridomas, mAbs were produced as ascites fluids and purified. By flow cytometric analysis, all mAbs recognized COP5 cells transfected with the full-length cDNA encoding hDCIR, but not mock-transfected cells (data not shown). Data presented in the present study were obtained with the anti-DCIR mAb 111F8 of isotype IgG1, kappa.

Results

Identification of the cDNA of DCIR

Alignment of amino acid sequences of type II membrane proteins of the calcium-dependent lectin family reveals a number of conserved motifs in the C-type lectin domain. A motif (SCYWFSH) shared between the hepatic ASGP Rs 1 and 2 and the macrophage lectin was used to search the Human Genome Sciences (HGS) databases using the tblastn algorithm (41), and a number of cDNAs were identified. Two sequences from DC libraries were selected and reanalyzed against the public and HGS libraries to detect homologous or identical cDNAs using the blastn algorithm. A single contiguous sequence (contig) of 1299 bp, composed of 5’ sequences from 14 different clones and containing an entire open reading frame, was isolated. A representative cDNA clone was sequenced in both strands to give the full-length sequence of the DCIR cDNA. This clone is 1272 bp in length (excluding the poly(A) tail) and contains a putative open reading frame of 711 bp (Fig. 1). The potential start codon is not in a consensus Kozak sequence (42) but because it is not preceded by an alternative ATG, and because stop codons exist at upstream positions, we predicted that this was the start of the encoded protein. A polypeptide of 359 aa was deduced from the sequence of the cDNA clone.

Separation of CD1a+ and CD14+ DC precursor subsets from cultured CD34+ cord blood cells was performed essentially as previously described (40). Briefly, after 6 days of culture in the presence of SCF, GM-CSF, TNF-α, and 5% human serum, cells were collected and labeled with FITC-conjugated OKT6 (CD1a; Ortho Diagnostic Systems, Raritan, NJ) and PE-conjugated Leu-M3 (CD14; Becton Dickinson). Cells were separated according to CD1a and CD14 expression into CD14+CD1a+ and CD14+CD1a- populations using a FACStar plus (Becton Dickinson). All the procedures of staining and sorting were performed in the presence of 0.5 mM EDTA to avoid cell aggregation. Reanalysis of the sorted populations showed a purity higher than 98%. Sorted cells were seeded in the presence of GM-CSF plus or minus TNF-α (1-2 x 105 cells/ml) for 6–7 additional days. Cells were routinely collected between day 11 and 14.

Immunohistological localization of DCIR positive cells

Microscope slides of acetone-fixed cryostat tonsil sections were incubated with 10 μg/ml anti-DCIR mAb 111F8 for 60 min, and subsequently with sheep anti-mouse IgG1 (The Binding Site, Birmingham, U.K.) followed by mouse anti-alkaline phosphatase-alkaline phosphatase (APAAP technique; Dako). Alkaline phosphatase activity was revealed using the Fast Blue substrate (Sigma). For double staining of DCIR and CD1a, tonsil sections were incubated with the mouse IgG1 anti-DCIR mAb 111F8 and the IgG2b anti-CD1a (Becton Dickinson) and then revealed by sheep anti-mouse IgG1 (The Binding Site, Birmingham, U.K.) followed by ExtrAvidin-peroxidase (Sigma). Alkaline phosphatase activity and peroxidase activity were respectively demonstrated using Fast Blue substrate and 3-amino-ethyl carbazole (Sigma).
that DCIR belongs to the type II Ca\(^{2+}\) binding domain. Analysis of the predicted amino acid sequence of DCIR indicated the presence of a single CRD (8, 44). The C-type lectin domain of DCIR has the greatest homology to the macrophage lectin (42%), the hepatic ASGPR-1 and ASGPR-2 (35% and 37%, respectively), and CD23 (32%) (Fig. 2A). The conserved cysteine residues (arrows in Fig. 2A) of the C-type lectin fold are clearly conserved across the members of this family; however, a number of distinguishing features can be seen. Like the macrophage and hepatic lectins, DCIR has a double cysteine motif at the start of the lectin domain (circle in Fig. 2A). The function of this supplementary cysteine is unknown, as there is apparently no other cysteine besides one indicated by a double circle. This residue may be involved in intermolecular disulfide bridge formation, although there is another cysteine in DCIR at position 91 (Fig. 1) that probably fulfills this function. The calcium-binding domain is conserved in DCIR including the poly(A) sequence, and contained a 5' UTR of 278 bp (data not shown). As for hDCIR, the putative start codon was not contained within a consensus Kozak region, but was preceded by an upstream stop codon, and the 3' UTR also contained sequences similar to hDCIR. The full-length clone was 1418 bp, estimated to be a full-length clone by sequence analysis, was selected and DNA sequenced. This clone showed features similar to hDCIR. The full-length clone was 1418 bp, excluding the poly(A) sequence, and contained a 5' UTR of 278 bp (data not shown). As for hDCIR, the putative start codon was not contained within a consensus Kozak region, but was preceded by an upstream stop codon, and the 3' UTR also contained sequences similar to rapid degradation signals, including four consensus ATTTA sites (data not shown). The predicted polypeptide sequence of DC94 (45) gives a clear demonstration of the differences in the two types of CRD, with notably a reduced loop at the position of the Ca\(^{2+}\) binding domain. Thus CD94 and the NKG2 receptors do not bind calcium and probably have an alternate ligand binding structure to that of the C-type lectins that fix calcium, DCIR included. A single potential N-glycosylation site is present at position 185 (Fig. 1). Phylogenetic analysis of the CRD domains (Fig. 2B) further indicates that DCIR is intermediate in structure between the group of macrophage/hepatic lectins and CD23 (Fig. 2B).

The analysis of protein sequence of DCIR (Fig. 1) predicts a 49-aa intracellular domain that contains a tyrosine at position 7 centered in the sequence ITYAEV that perfectly matches the consensus sequence I/VXYXXL/V of the ITIM (28–30, 46, 47). This type of domain has been shown to act as an inhibitory motif in molecules of the lectin family such as human NKG2A/B (24) and murine Ly49 (48, 49).

Identification of a mouse homologue of hDCIR

Bioinformatics searches in the EST databases (GenBank dbEST) using the predicted polypeptide sequence of hDCIR (tblastn algorithm) revealed mouse clones encoding a homologous protein. One IMAGE consortium (LLNL) cDNA clone AA170532 mouse spleen (50), estimated to be a full-length clone by sequence analysis, was selected and DNA sequenced. This clone showed features similar to hDCIR. The full-length clone was 1418 bp, excluding the poly(A) sequence, and contained a 5' UTR of 278 bp (data not shown). As for hDCIR, the putative start codon was not contained within a consensus Kozak region, but was preceded by an upstream stop codon, and the 3' UTR also contained sequences similar to rapid degradation signals, including four consensus ATTTA sites (data not shown). The predicted polypeptide is 238 residues in length and codes for a type II membrane protein with an ITIM and a C-type lectin domain. Alignments with the human protein (Fig. 3) showed 54% identity, 65% homology over the whole sequence. Notably, the ITIM domain is localized within a highly conserved region (13 of 15 residues are identical). There are...
three potential N-glycosylation sites at positions 91, 131, and 136.

Of interest is the conserved membrane-proximal glutamine motif (FQKYSQLLE), and the cysteine residue potentially implicated in disulfide bridge formation (boxed in Fig. 3). Equally, the C-type lectin domains show blocks of conservation, including the EPS motif at positions 197–199. Differences seen between hDCIR and the human macrophage and hepatic lectins are retained in the mouse sequence, notably phenylalanine (F) instead of tryptophan (W) at position 167, glutamine (Q) at positions 179 and 183, the addition of tryptophan-glycine (WG) at positions 216–217, and serine (S) instead of tryptophan at position 229 (filled circles in Fig. 3). We thus propose that this clone is the mouse homologue of hDCIR.

**hDCIR is coded for by a single copy gene localized on chromosome 12**

Southern blot analysis of genomic DNA with a probe consisting of a 391-bp DNA fragment from the 3′ end of DCIR cDNA showed a single band at ~7 kb after digestion with the enzymes BamHI and SaeI (data not shown). These data indicate a unique gene for DCIR. Chromosomal localization with the Stanford G3 panel gave as closest marker SHGC-12041, with a lod score of 7.7. This marker, which is the gene coding for M130 Ag/CD163 (51), is localized to chromosome 12p13. This region is host to a number of genes encoding receptors of the C-type lectin-like family, notably CD94, the NKG2 molecules, CD69, AICL, and NKR-P1 that belong to the NK gene complex (15, 16).

**DCIR is expressed in immune tissues**

Northern blot analysis (Fig. 4) of DCIR showed a band at 1.3 kb. This band is slightly smeared and may indicate the presence of a second mRNA at 1.5 kb. The high stringency washes used for this Northern blot tend to suggest that this is not due to a related gene, but rather to the presence of two transcripts. mRNA was detected in greatest quantity in peripheral blood leukocytes, with moderate quantities in spleen, lymph node, and bone marrow, and at very low levels in thymus (Fig. 4) as well as in spinal cord and trachea (data not shown). However, DCIR mRNA was not detected in heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, prostate, testis, ovary, small intestine, colon, stomach, and thyroid (data not shown). Taken together, these data indicate a preferential expression of DCIR in hematopoietic tissues.

**DCIR expression is restricted to myelo-monocytic cells and B cells**

As DCIR was expressed preferentially in immune tissues, we further investigated its cellular distribution by RT-PCR analysis in various myelo-monocytic and lymphoid cell types. As shown in Fig. 5A, DCIR mRNA could be amplified from DC generated in vitro by culturing CD34+ cord blood progenitors for 12 days in the presence of GM-CSF plus TNF-α (35). Expression was also detected in macrophages and granulocytes derived from CD34+ progenitors in the presence of M-CSF or G-CSF, respectively. This suggests that DCIR is expressed in immune tissues.
expression did not appear to alter in response to treatment with PMA/ionomycin. In freshly isolated cells, DCIR was expressed in PBMC and strongly detected in blood monocytes, whereas a low expression was seen in tonsillar B cells. Interestingly, DCIR mRNA was not detected in purified blood T cells nor in blood NK cells (Fig. 5A). Furthermore, DCIR mRNA could not be detected in a number of cell lines including U937, HL60, THP1 (myelomonocytic cells), JY, RAMOS, DAUDI, BL2 (B cell lines), Jurkat and MOLT4 (T cell lines), TF1 (erythro-leukemia), CHA (kidney carcinoma), MRC5 (fetal lung fibroblasts) and SW620, and HT29 (colon carcinoma cell lines) (data not shown).

The expression of DCIR was further studied by flow cytometry analysis of peripheral blood leukocytes after staining with a specific mAb recognizing the DCIR extracellular domain (Fig. 5B). DCIR was found to be expressed by all circulating CD14+ monocytes and, at lower intensity, by the majority (88 ± 5%, n = 3) of CD19+ B lymphocytes. Flow cytometric analysis showed strong expression of DCIR by freshly isolated CD15+ blood granulocytes (Fig. 5B), thus confirming the expression of DCIR mRNA observed on in vitro-derived granulocytes (Fig. 5A). Finally, DCIR could not be detected on the surface of circulating CD3+ T cells nor on CD16+ or CD56+ NK cells (Fig. 5B).

**In vitro, DCIR is differentially expressed on DC subsets and its expression is regulated during DC maturation**

Human DC generated in vitro from cord blood CD34+ progenitors represent an heterogeneous population of cells that arise from different precursors and possess different functions (34, 52, 53). Because DCIR mRNA was detected in total CD34+ -derived DC at day 12 (Fig. 5A), we further analyzed its distribution on different DC subsets and activation states. By two-color flow cytometry (Fig. 6A), DCIR was found to be expressed on both CD14+ and CD1a+ DC precursor cells obtained by culturing cord blood CD34+ progenitor cells for 5–6 days with GM-CSF and TNF-α in the presence of human serum (34). The intensity of DCIR expression was generally stronger on the CD14+ subset of cells. To determine whether DCIR expression is equally affected by further differentiation of these two precursors into DC, CD1a+/CD14+ and CD1a-/CD14+ cells were flow cytometry-sorted at day 6 of culture and further cultured for 6 days in the presence of GM-CSF with or without TNF-α. As shown in Fig. 6B, CD14+ -derived DC expressed considerably higher levels of DCIR than their CD1a+-derived counterparts. Analysis of DCIR mRNA expression by RT-PCR also confirmed the lower levels of DCIR mRNA in CD1a+-derived DC (Fig. 6C). Notably, for both subsets, DCIR expression was significantly higher upon removal of TNF-α, between days 6 and 12 (Fig. 6B).

Human DC can be also generated in vitro by culturing blood monocytes with GM-CSF and IL-4 (36, 54, 55). As shown in Fig. 7A, monocyte-derived DC strongly express DCIR after 7 days of culture with GM-CSF and IL-4, indicating that DCIR expression is maintained during the differentiation of monocytes into DC. At this stage monocyte-derived DC are considered to be immature as they express low levels of CD80, CD86, MHC class II molecules, CD25, and CD83 (Ref. 4 and data not shown). Of interest, DCIR expression decreased when these cells were further cultured with LPS or CD40 ligand (Fig. 7A), two signals that induce DC activation and maturation (4). The down-regulation of DCIR expression was also observed at the mRNA level, as shown in Fig. 7B in the case of CD40 activation, where DCIR mRNA expression was totally lost by 24 h of coculture with CD40 ligand-expressing L cells. It should be noted that DCIR mRNA expression could not be altered by treatment of the same cells with a combination of PMA and Ionomycin (Fig. 7B).
Expression of DCIR in vivo

To determine the pattern of expression of DCIR in vivo, we performed immunohistological studies. In tonsil, DCIR-positive cells were found beneath the epithelium and around the B cell follicles, but not observed in germinal centers (Fig. 8, A and B). A weaker expression was seen in cells of the mantle zone of the follicles, principally composed of naive B cells (Fig. 8B). At higher magnification (Fig. 8C), cells that strongly express DCIR display a dendritic morphology. Double staining of DCIR and CD1a (Fig. 8D) showed that the Langerhans-like cells in the tonsil epithelium that are stained with anti-CD1a mAb are not stained with anti-DCIR mAb, whereas DCIR-positive (CD1a negative) cells are mostly present within the subepithelial region.

Discussion

In the present study, we have reported the cloning and characterization of DCIR, a novel member of the C-type lectin superfamily. DCIR is a type II membrane protein closest in homology to the macrophage lectin and to the hepatic ASGPR-1 and -2. By Northern blot analysis, DCIR mRNA was detected only in immune tissues, and both RT-PCR and flow cytometry analysis have demonstrated a restricted expression of DCIR on APC (DC, monocytes, macrophages, and B cells), as well as on granulocytes. Focusing our study on DC as APC, we have shown that DCIR is expressed by DC generated in vitro by culturing either cord blood CD34⁺ progenitors or peripheral blood monocytes. DCIR expression is particularly strong on CD34⁺/CD14⁻/CD1a⁺ precursors and on DC obtained from monocytes. Notably, both of these populations display features resembling interstitial/dermal-type DC (34, 53, 56). In contrast, DC derived from CD1a⁻/CD14⁻ cells that represent precursors of epidermal/Langerhans cells (34) express low levels of DCIR. These data are in agreement with the in vivo distribution of DCIR showing that an anti-DCIR mAb did not stain CD1a⁻ Langerhans-like cells in tonsil epithelium (Fig. 8) or immature Langerhans cells in skin epidermis (data not shown). DCIR expression can be decreased by signals inducing DC maturation (e.g., CD40 ligand, LPS, and TNF-α) that would correspond to a stage subsequent to migration into secondary lymphoid tissues in response to proinflammatory factors and to contact with Ag-specific T cells expressing CD40 ligand. Taken together, these results indicate that DCIR is selectively expressed on DC subpopulations according to their origin and stage of maturation. Additional experiments will be required to gain insight into the functional significance of this particular expression pattern of DCIR.

The chromosomal localization of hDCIR close to the NK gene complex on chromosome 12p13 is interesting, particularly as DCIR was not detected in NK and T cells. The complex of C-type lectin CRDs is only partially conserved between DCIR and other C-type lectin CRDs. The CRD of DCIR thus displays the closest homology with the hepatic ASGPR-like lectin family members at this locus includes CD69, AICL, CD161, CD94, and the NKG2 family (15, 16, 57), as well as the recently described MAFA-like receptor gene (58). In the mouse the NK gene homologue. Although the hDCIR gene maps close to the NK gene complex, exemplified by the Ly49 lectin-like family, is localized to the syntenic region on chromosome 6 (59); thus, it will be of interest to determine the localization of the DCIR mouse gene homologue. Although the hDCIR gene maps close to the NK complex, the sequence encoding the extracellular carboxyl-terminal portion of the molecule is not typical of the NK cell receptors. Characteristics of the C-type lectin CRDs are only partially conserved in the NK cell receptors, with a partial or total lack of the Ca²⁺-ligating residues that are associated with sugar-binding sites (17). In striking contrast, the Ca²⁺-ligating residues are well conserved between DCIR and other C-type lectin CRDs. The CRD of DCIR thus displays the closest homology with the hepatic ASGPRs. Yet, the localization of the latter genes on chromosome 17 (60) could suggest that DCIR represents an evolutionary intermediate between the NK cell receptors and the hepatic lectins.

The cytoplasmic domain of DCIR contains one ITIM motif (ITYAEV). This type of domain has been shown to inhibit signal transduction via activation receptors (e.g., ITAM-bearing receptors) and is present in the cytoplasmic tail of C-type lectin-like
molecules including the human NKG2A/B and murine Ly49 family members expressed by NK cells (18, 59). Inhibition is mediated by the recruitment of SHP-1 (SH2-containing phosphatase-1) and/or SHP-2 phosphatases to the phosphorylated ITIM domain (28). By analogy to these known inhibitory receptors, DCIR may be implicated in inhibition of molecular events associated with cellular activation. It is proposed that receptors with two cytoplasmic tyrosine motifs, like NKG2A/B and the Ig-like superfamily members KIR or gp49, may interact simultaneously with the tandem SH2 domains of SHP-1, thereby producing a more stable interaction, whereas receptors with a single tyrosine motif such as mouse Ly49A may form homodimers for recruiting SHP-1 (46, 61). The presence of a single ITIM in the intracytoplasmic tail of DCIR may indicate that this molecule also forms a dimer (as suggested by the presence of a supplementary cysteine in its extra-cellular domain) to recruit SHP-1 or SHP-2.

The presence of a putative inhibitory motif in the cytoplasmic tail of DCIR and its expression on monocytes and some immature DC subsets suggest that DCIR may play a role in modulating DC differentiation and/or maturation. This has been recently described for the p40/LAIR1 molecule, an ITIM-containing receptor of the Ig-like superfamily (62), the cross-linking of which blocks GM-CSF-induced differentiation of mature monocytes and CD34+ peripheral blood precursors into DC (63). DCIR may also be involved in Ag uptake by immature DC because the consensus for Ag internalization through receptor-mediated endocytosis. Following cross-linking, DCIR was only slowly and weakly internalized in monocyte- and CD34+ derived DC, in contrast to the rapid kinetics observed with the MMR (data not shown). This finding suggests that Ag capture by receptor-mediated endocytosis is not the principal function of DCIR.

The expression of DCIR on B lymphocytes also appears to be regulated according to their stage of activation. We found that while the majority of blood B cells express relatively high levels of DCIR, only a subset of freshly isolated tonsilar B cells were stained with anti-DCIR mAb, and at lower intensity (data not shown). Furthermore, two-color flow cytometry demonstrated that DCIR+ tonsilar B cells coexpressed CD44 but not CD38 (data not shown), two markers that discriminate germinal center B cells (CD38+/CD44−) from naive and memory B cells (CD38−/CD44+) (70). In keeping with this, anti-DCIR mAb did not stain germinal center B cells on tonsil sections, but weakly stained B cells within the mantle zone of the B follicles (Fig. 8). These data indicate that DCIR is mostly expressed by resting naive and memory circulating B cells and suggest that DCIR expression is down-regulated by activation. It is interesting to note that the expression of DCIR on B cells parallels that of ILT2, an Ig-like superfamily member containing ITIM motifs, that has been shown to inhibit B cell activation through the B cell Ag receptor (71). We are presently investigating whether DCIR may also negatively regulate B cell activation.

The natural ligand(s) for DCIR have not been yet identified. Carbohydrate ligands are likely because DCIR belongs to the C-type lectin superfamily. The amino acid sequence present in the CRDs of animal lectins provides information about saccharide-binding specificity. For example, the sequence EPN is found in CRDs known to bind mannos or glucose derivatives, and is present in two domains of the MMR (8). In contrast, the sequence...
QPD is characteristic of CRDs that bind galactose and N-acetyl-
galactosamine and is present in the hepatic ASGPR-1 and -2 (8).
Both human and mouse DCIR display a variant motif (EPS) in
place of EPN or QPD. An EPS sequence is present in the CRD-2
of the MMR but is believed to contribute only weakly to binding
of polyvalent ligands (72).

In conclusion, both human and mouse DCIR are type II proteins
containing a consensus ITIM in their intracytoplasmic tail. hDCIR
is encoded by a gene localized close to the NK complex on chro-
mosome 12. Unlike the type II lectin-like members of this com-
plex, hDCIR contains a bona fide extracellular lectin CRD with
calcium-binding residues. Moreover, unlike immunoreceptors
of the NK complex, hDCIR is not expressed by NK and T cells.
DCIR may thus represent a molecule intermediate in structure and
function(s) between the NK cell receptors and typical CRD-bear-
ing lectins such as the ASGPRs that function in ligand internal-
ization. The expression of DCIR on discrete DC subsets and mat-
uration stages strongly suggests a particular role in directing the
ontogeny and/or the Ag-handling potential of this pivotal cell for
initiation of specific immunity.

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FIGURE 8. Expression of DCIR on tonsil tissue sections. A, At low magnification, DCIR (blue) is strongly expressed by isolated cells beneath the
epithelium (Ep) and throughout the tonsil except in the germinal center (GC). B, A weaker expression is seen in cells of the mantle zone of the follicle.
C, At higher magnification, strong expression is seen in large dendritic-like cells outside of the GC (arrow) and weak expression is seen in smaller cells
in the surrounding tissue. D, Double staining of DCIR (blue) and CD1a (red). Langerhans-like cells in the epithelium are stained with anti-CD1a mAb but
not by anti-DCIR mAb, whereas DCIR-positive cells are mostly present within the subepithelial region. Original magnifications: ×40 (A); ×200 (B and
D), and ×1000 (C).

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