Chapter 7
Dendritic Cell Immunoreceptor (DCIR): An ITIM-Harboring C-Type Lectin Receptor

Tomonori Kaifu and Yoichiro Iwakura

Abstract C-type lectin receptors (CLRs) have been recognized as one of the pattern recognition receptors that trigger immune responses against pathogens. Dendritic cell immunoreceptor (DCIR) is a type II membrane protein that contains an extracellular carbohydrate recognition domain (CRD) and a long cytoplasmic tail with an immunoreceptor tyrosine-based inhibition motif (ITIM). Only one molecular species is identified in humans (DCIR), while four family molecules (DCIR1-4) are present in mice. Human DCIR and mouse DCIR1 dampen immune responses through ITIM-mediated reaction. DCIR binds mannose and fucose and also pathogenic organisms, but the structure of the ligand carbohydrates still remains to be determined. DCIR1 is important for the homeostasis of the immune system, and the deficiency causes autoimmune diseases. DCIR also acts as an attachment factor for HIV in dendritic cells and HIV-infected T cells. DCIR1 is also implicated in the pathogenesis of mosquito-transmitted virus and protozoan infections. This chapter highlights the roles of human and mouse DCIR in immune responses and immune homeostasis revealed by in vitro cell-based studies as well as in vivo gene-depleted mouse analyses.

Keywords C-type lectins • HIV • Phosphatases • ITIM • Homeostasis • Bone metabolism

A large array of membrane receptors is expressed on professional antigen-presenting cells (APCs) to sense pathogen invasions for activating innate immunity and to present foreign peptides on major histocompatibility complex II (MHC-II) or MHC-I for activating adaptive immunity. DCs and macrophages express plentiful germline-encoded innate receptors to recognize pathogen-associated molecular patterns (PAMPs) of invading pathogens and conserved molecular structures of pathogens. These receptors are called as pattern recognition receptors (PRRs). Toll-

T. Kaifu (✉) • Y. Iwakura
Research Institute for Biomedical Science, Division of Experimental Animal Immunology, Center for Animal Disease Models, Tokyo University of Science, 2669 Yamazaki, Noda, Chiba, Japan
e-mail: kaifu@rs.tus.ac.jp

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like receptors (TLRs) and C-type lectin receptors (CLRs) are membrane-embedded PRRs that response to multiple types of PAMPs (van Vliet et al. 2007). Although TLRs are able to stimulate maturation of DCs through recognition of different types of PAMPs, they do not mediate antigen uptake to present peptides on MHC-I or MHC-II. While CLRs detect diverse PAMPs to synthesize pro- and anti-inflammatory mediators, they can incorporate pathogens into cytoplasm to facilitate antigen presentation (Bergman et al. 2004; Hunger et al. 2004).

There is a diverse group of proteins in C-type lectin family. C-type lectins were originally characterized by their abilities to bind carbohydrates. Soluble and membrane-bound forms function as C-type lectin proteins, but they have a common domain, termed the carbohydrate recognition domain (CRD), which contains disulfide bridges between cysteine residues. C type is termed by the property that requires Ca^{2+} for binding carbohydrate structures, but some C-type lectin receptors bind their ligands in a Ca^{2+}-independent manner. Moreover, they recognize multiple types of molecules, proteins, and lipids, other than carbohydrates. Whereas a majority of CLRs recognize carbohydrate structures on pathogens, some are found to interact with self-glycoproteins or endogenous ligands.

A group of immune receptors is consisted of counterbalancing two types of receptors: one mediates upregulation of cellular activities and the other counter-regulates the activation induced by these receptors. The former type of receptors contains an immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic domain. Another type of receptors in this group, with a short cytoplasmic tail and a charged amino acid residue in the transmembrane region, forms a complex with an adaptor protein containing ITAM, such as Fc receptor γ-chain. On the other hand, counterbalanced receptors that are capable of dampening activation signal contain immunoreceptor tyrosine-based inhibitory motif (ITIM), which provides a docking site for phosphatases to dephosphorylate phosphotyrosine residues in signaling molecules or ITAMs. Thus, it is suggested that the balance of signals between ITAM-harboring and ITIM-harboring receptors determines the adequate cellular conditions, and interruption of the delicate signal balance causes harmful havoc, such as excessive inflammation, self-tissue damages, and autoimmune diseases. So far, two CLRs have been identified as an ITIM-harboring receptor, and dendritic cell immunoreceptor (DCIR) is one of the best studied receptors. In this review, we will introduce its expression pattern, intracellular signal pathways, and physiological roles at the levels of cells and animals.

7.1 The *DCIR* Gene Is Located in the CLR Cluster

Cloning of the human and mouse DCIR gene has been reported in four reports. (1) Human DCIR was identified by the homology search for the conserved seven amino acid sequences of the CRD in hepatic asialoglycoprotein receptors (ASGPRs) and macrophage lectin in the Human Genome Sciences databases. Amino acid identity of the deduced CRD domain of human DCIR is 42 %, 35 %,

and 37 % for macrophage lectin, ASGPR1, and ASGPR2, respectively. Mouse DCIR1 molecule was also identified in the EST databases, using the deduced protein sequence of human DCIR. The predicted protein sequence of mouse DCIR is 54 % identical to human DCIR (Bates et al. 1999). (2) Human DCIR gene was cloned by subtractive cloning of differentially expressed cDNA from human monocyte-derived DCs, which were treated with or without keyhole limpet hemocyanin. The gene was named lectin-like immunoreceptor (LLIR) gene in that study. The predicted protein sequence of the LLIR is highly similar to ASGPRs in the BLAST search. The LILR gene is located in the chromosome 12p13 locus, close to NK cell receptors clusters that encode multiple lectin-like NK receptors. Two shorter variants were isolated from human myeloid cell line, which lacked the nucleotide sequence encoding the transmembrane region (Huang et al. 2001). (3) Upregulation of *DCIR* mRNA was shown in GM-CSF-treated human neutrophils compared with non-treated neutrophils using differential display polymerase chain reaction method. The gene was assigned as C-type lectin superfamily 6 (*CLECSF6*). A homologous BAC clone in nucleotide sequence databases was assigned in chromosome 12p13.1 (Richard et al. 2002). (4) Yeast signal sequence trap screening identified cDNA of mouse DCIR1 from mouse bone marrow-derived DCs, which were induced maturation by TGF-β1. The predicted amino acid sequence of the CRD region of mouse DCIR1 was 48 % identical to Dectin-2 (Kanazawa et al. 2002). Meanwhile, homology search for the rat *Dcir* to various human and mouse C-type lectin-like receptors identified only one *DCIR* gene located in the chromosome 12 in humans and four genes for mouse DCIR (*Dcir1-4*) in the chromosome 6 at the telomeric region near the NK cell receptor clusters (Kerscher et al. 2013; Flornes et al. 2004). Human DCIR and mouse DCIR1 have 55 % amino acid sequence identity, and mouse DCIR1 is 74 %, 57 %, and 52 % identical to DCIR2, DCIR3, and DCIR4, respectively. In this review, we use mouse DCIR as mouse DCIR1 hereafter.

7.2 *DCIR* Gene Is Expressed in Cells in the Immune System

Human *DCIR* mRNA is highly detected in peripheral blood leukocytes and moderate in bone marrow, spleen, and lymph node, while the *DCIR* mRNA level is low in the thymus, spinal cord, and trachea. *DCIR* mRNA is observed in DCs, macrophages, and granulocytes, which are induced in vitro from $CD34^+$ cord blood progenitors as well as freshly isolated blood monocytes. *DCIR* mRNA is slightly detected in tonsil B cells, but not in blood T cells and NK cells. Peripheral blood neutrophils express a low level of *DCIR* mRNA. In accordance with the presence of *DCIR* mRNA, DCIR protein is detected in peripheral blood $CD14^+$ monocytes, $CD15^+$ granulocytes (including neutrophils, eosinophils, and monocytes/macrophages), and $CD19^+$ B cells, but not in $CD56^+$ NK cells and $CD3^+$ T cells. In immunohistochemical staining, the expression of DCIR in the tonsil is found in the broad area below the epithelium and weakly in the mantle zone of the follicle, but

not in the germinal center. DCIR-positive cells have a dendritic shape and are negative for CD1a, which is a maker for Langerhans cells in the epithelium (Bates et al. 1999). Thus, human DCIR is expressed in myeloid cells and APCs including B cells, and the expression pattern is distinct among subsets of DCs.

The expression pattern of mouse *Dcir* is similar to humans. Mouse *Dcir* mRNA is high in the spleen and lymph nodes, in which APCs, such as DCs, macrophages, and B cells, accumulate in the resting and inflammatory conditions. *Dcir* mRNA is detected in bone marrow-derived DCs but not in NK cells. Consistent with the expression profiles of *Dcir* mRNA, mouse DCIR was detected on the surface of splenic B220+ or CD19+ cells, CD11c+ cells, and Mac1+ cells (Kanazawa et al. 2002). Indeed, all the DCIR-expressing cells express MHC class II, indicating that mouse DCIR is exclusively expressed on APCs. However, mouse DCIR expression in neutrophils still remains undetermined. Mouse *Dcir2* mRNA is observed in macrophages, DCs, neutrophils, B cells, and NK cells. At present, mouse DCIR2 is used as a specific marker for CD8– DCs (Dudziak et al. 2007). Mouse *Dcir3* mRNA is detected in macrophages, DCs, neutrophils, and B cells, while mouse *Dcir4* mRNA is in macrophages, DCs, and neutrophils (Flornes et al. 2004). The precise expression patterns of mouse *Dcir3* and mouse *Dcir4* remain yet unclear.

7.3 DCIR Is One of the ITIM-Harboring C-Type Lectin Receptors

The deduced amino acid alignment displays human DCIR as a type II receptor of 237 aa with a single CRD in the extracellular C-terminus portion and ITIM in the intracellular N-terminus portion. Human DCIR carries no signal sequence but a putative hydrophobic signal anchor sequence. A number of conserved cysteine residues remain in the CRD of human DICR, in comparison with ASGPRs and macrophage lectin. Human DCIR forms disulfide bonds with a pair of cysteines at aa positions 106 and 117, 134 and 230, and 203 and 222 (UniPortKB; Q9UMR7). The CRD has the conserved calcium-binding domain consisted of an EPS motif (at position 195–197), glutamate (at position 201), and asparagine and aspartate (at positions 218 and 219) (Bates et al. 1999). The calcium-binding motif is conserved in ASGPRs and macrophage lectin, although they have QPD motif instead of EPS motif. It is noteworthy that the NKG2 receptors, which are Ca2+-independent C-type lectin receptors mainly in NK cells, lack the motif in the amino acid alignment. Crystal structure analysis of CD94 revealed the lack of the corresponding loop at the position of the calcium-binding domain (Boyington et al. 1999). Human DCIR has a potential consensus N-glycosylation site at position 185, which is suggested to have a hindrance effect on binding with DCIR ligands (Bloem et al. 2013).

The amino acid sequence of human DCIR displays the intracellular domain with 48 amino acids, containing ITYAEV at the position from residue 5–10. The sequence matches the canonical ITIM, an inhibitory signal motif whose conserved sequence is S/I/V/LxYxxI/V/L (Ravetch and Lanier 2000). The signal motif has been shown to act as a docking site for phosphatases that counterbalances cellular activities through inhibition of a cascade of tyrosine kinases.

Two mouse homologues have structural features similar to human DCIR. Mouse DCIR and DCIR2 are type II protein with a single CRD, conserved cysteine residues, and the ITIM sequence. They also have potential cysteine residues that are supposed to be involved in disulfide bond formation. Mouse DCIR has conserved EPS motif (at position 197–199), glutamate (at position 203), and asparagine and aspartate (at positions 219 and 220), but DCIR2 has EPN motif, which is thought to be responsible for mannose binding. DCIR has three potential N-glycosylation sites, whereas DCIR2 has four potential N-glycosylation sites. In contrast, DCIR3 and DCIR4 lack the consensus ITIM sequence, although a tyrosine residue remains at the corresponding region. Asparagine in the calcium-binding domain of DCIR3 and DCIR4 is replaced by serine, and aspartate of DCIR4 is substituted for valine, suggesting that they may function in a Ca2+-independent way (Flornes et al. 2004).

Human DCIR is a unique C-type lectin receptor with a long cytoplasmic tail and an ITIM, similarly to Clec12a. The same structural features are conserved in mouse DCIR and DCIR2. In B-cell lymphoma lacking Fc receptors, chimeric proteins consisting of the extracellular region of FcγRIIB and the cytoplasmic of mouse DCIR dampened B-cell receptor (BCR)-mediated Ca2+ flux and total tyrosine phosphorylation. The inhibitory effect of DCIR depends on the tyrosine residue in the ITIM (Kanazawa et al. 2002). The peptide covering the ITIM region of human DCIR that contains phosphorylated tyrosine binds to SHP-1 and SHP-2, but not SHIP, upon incubation with human neutrophil lysates (Richard et al. 2006) (Fig. 7.1).

The expression of DCIR in DCs implies inhibitory roles in maturation, secretion of soluble mediators, and antigen presentation. Human pDCs enriched by anti-BDCA-4 magnetic beads from peripheral blood expressed BDCA-2 and DCIR but not DC-SIGN, mannose receptor, or Dectin-1. Induction of DCIR-mediated signal by cross-linking with anti-DCIR antibody reduced IFN-α production in response to TLR9 ligands, whereas DCIR triggering did not affect the expression levels of co-stimulatory molecules in TLR9-activated pDCs (Meyer-Wentrup et al. 2008). Thus, DCIR in DCs appears not to be involved in the regulation of antigen recognition and co-stimulation in the process of antigen presentation. Many of C-type lectin receptors on DCs are known to function as an endocytic receptor and internalize bound ligands (Figdor et al. 2002). DCIR activation with antibody causes internalization of DCIR in a clathrin-dependent manner. This may explain the mechanism of the inhibitory effect of DCIR on TLR9-induced IFN-α production, which locates in endosomal compartments. However, it still remains to be elucidated whether the ITIM motif of DCIR is required for the downregulation of cytokine production and whether the phosphatases (SHP-1 and SHP-2) are involved

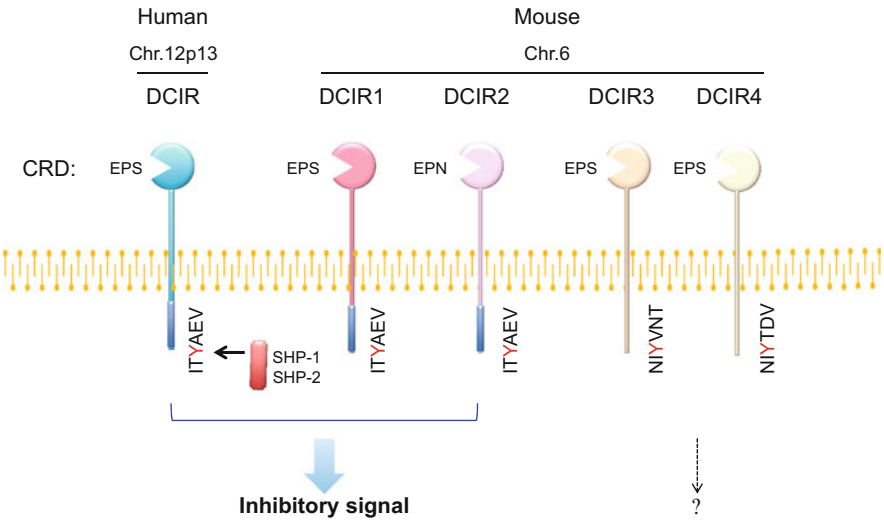

Fig. 7.1 Structures of human DCIR and mouse DCIR1, DCIR2, DCIR3, and DCIR4. Dendritic cell immunoreceptor (DCIR) is a C-type lectin receptor with the ITIM in the cytoplasmic region. The genes for human and mouse DCIR are located in chromosomes 12p13 and 6, respectively. Four genes are identified in mice. The CRD of human DCIR and mouse DCIR1, DCIR3, and DCIR4 has EPS motif that is important for Ca2+-dependent carbohydrate recognition, but mouse DCIR2 has EPN motif. Human DCIR, mouse DCIR1, and mouse DCIR2 have the canonical sequence of ITIM and mediate inhibitory signals through SHP-1 and SHP-2. In contrast, mouse DCIR3 and DCIR4 lack the consensus ITIM sequence. They have a tyrosine residue in the cytoplasmic part, but it is not yet known whether they transduce intracellular signal. CRD: carbohydrate recognition domain. ITIM motif: S/I/V/LxYxxI/V/L. E: glutamic acid, P: proline, N: asparagine, S: serine

in the signaling components downstream of TLR9. In addition, DCIR inhibits TLR8-induced pro-inflammatory cytokine production from human monocyte-derived DCs (moDCs). DCIR triggering by specific antibodies reduced the production of TNF-α and IL-12 from moDCs upon stimulation with a ligand for TLR8 but not ligands for TLR3, TLR4, and TLR2/Dectin-1. This is probably explained by the fact that DCIR is internalized into LAMP-1-positive lysosome compartments and TLR8 and TLR9, but not TLR3, TLR4, or TLR2/Dectin-1, are also present in endosomal/lysosomal compartment (Meyer-Wentrup et al. 2009; Kawai and Akira 2008; Kawasaki and Kawai 2014). There is no evidence of the crosstalk between mouse DCIR and TLRs in mouse APCs. Nonetheless, these results indicate the importance of DCIR-mediated inhibition in limiting and modifying DC-associated immune responses.

7.4 DCIR Interacts with Carbohydrates and Ligands on Pathogens

Cell surface-embedded receptors such as TLR and immunoglobulin-like receptors trigger downstream signaling cascades to exert their functions after engagement with their ligands. CLRs bind not only to sugar moieties of membrane components of invading pathogens but also to endogenous proteins and glycolipids (Zhang et al. 2012; Ahrens et al. 2012; Ishikawa et al. 2009). To understand the molecular basis of DCIR-mediated signals and its function, it is important to elucidate the carbohydrate structures of the ligands. Glycan array data of the Consortium for Functional Glycomics (CFG) shows that human DCIR-Fc protein binds to sulfated lactose (Lac), LacNAc, and alpha-1-acid glycoprotein (AGP), which contains biantennary N-glycans (Hsu et al. 2009). The glycan array data of CLRs, including DCIR, are available from the CFG database. The human DCIR-Fc protein does not bind polysaccharide extract from a fungus, *Ganoderma lucidum*, analyzed by enzyme-linked immunosorbent assay. CLRs containing EPN motif, such as mannose-binding protein (MBP) and macrophage mannose receptor (MMR), are known to bind mannose (Man) and fucose (Fuc). Human DCIR-Fc protein, which has EPS motif in the CRD, binds not only Man and Fuc but also galactose (Gal) and GalNAc, although the binding affinities are lower than those of Man and Fuc (Lee et al. 2011). Human DCIR-Fc protein binds Lewis^b and Man_3 glycans, and truncation of N-glycosyl carbohydrate chains in the CRD increases the binding affinity for these glycans. However, the truncated and non-truncated forms of human DCIR expressed on CHO cells could not bind Lewis^b and Man_3 glycans. These binding assays using Fc-fusion protein reveal that human DCIR has a potential to bind a variety of sugar chains.

Tyrosine phosphorylation of human DCIR was increased when it was expressed in mutant CHO cells that lacked DCIR ligands compared to parent CHO cells, suggesting that *cis* interaction of DCIR with its ligands may affect the phosphorylation status of the ITIM (Bloem et al. 2013). Further analysis showed that human DCIR-Fc protein with truncated form of N-glycans binds Lewis^a and sulfo-Lewis^a, but human DCIR-Fc protein with N-glycan does not bind the glycans. Human DCIR-Fc binds to keratinocytes, gastric cancer cells, colon carcinoma cells, and prostate cancer cells as well as helminths such as *S. mansoni* and *T. spiralis* (Bloem et al. 2014). Moreover, DCIR-Fc fusion protein weakly binds commensal microbiota (Hutter et al. 2014). The structure of the DCIR ligands on microbiota is unclear, but probably a minor population of commensal microbiota expresses DCIR ligands. These data support the idea that DCIR recognizes both endogenous and pathogenic ligands.

Intravenous immunoglobulin (IVIg) therapy is widely used as a therapy for autoimmune diseases and inflammatory diseases. Although the action mechanism of IVIg remains controversial, sialylated immunoglobulins (SA-IgGs) are suggested to mediate the IVIg through SING-R1 (the murine homologue of DC-SIGN) in the treatment of arthritis and thrombocytopenic purpura in mice as

well as through CD22 (Siglec-2) in promoting apoptosis of human B cells (Anthony et al. 2008; Kaneko et al. 2006; Seite et al. 2010). SA-IgG also ameliorates ovalbumin-induced airway hyperresponsiveness (AHR) in mice by inducing Foxp3+ Treg cells through induction of tolerogenic DCs (Massoud et al. 2012). In this model, the effect of SA-IgG depends on DCIR in DCs. In immunofluorescence analysis, SA-IgG is fluorescently colocalized with DCIR, and DCIR-expressing CHO cells are stained with SA-IgG, but not with non-SA-IgG, indicating that DCIR serves an important role in SA-IgG-induced inhibition (Massoud et al. 2014). However, the direct binding between DCIR and sialic acid on IgG has not yet been proved.

7.5 DCIR Is an Attachment Factor for HIV and HCV

Human DCIR is implicated in the attachment and transmission of HIV in DCs and CD4+ T cells. Attachment of HIV to DCs is known as one of the entry routes to establish infection to CD4+ T cells. The interaction of viral envelope glycoproteins with CLRs, such as DC-SIGN, MR, and langerin, contributes to the attachment of HIV to DCs and transfers the virus to CD4+ T cells or macrophages (Turville et al. 2001, 2002; de Witte et al. 2007). Then, viral envelope protein binds CD4 protein and coreceptors such as CXCR4 or CCR5 on T cells and macrophages, causing membrane fusion of virus, following penetration into these cells. Knockdown of DCIR expression with siRNA or treatment with antibodies against DCIR in human immature moDCs decreased HIV transmission to autologous CD4+ T cells. Raji-CD4+ T cells that was transfected with *DCIR* gene remarkably augmented the binding and viral production, while Raji-CD4+ T cells expressing neck domain-deficient human DCIR failed to promote HIV binding (Lambert et al. 2008). As the stalk region of CLRs is responsible for multimerization, this result suggests that the formation of dimeric DCIR is necessary for efficient recognition of possible DCIR ligands on HIV (Jin et al. 2014). When the efficiency of HIV attachment and transfer compares that of DC-SIGN which can form tetramer, DCIR is less efficient than DC-SIGN (Jin et al. 2014). Under the physiological conditions, T cells do not express DCIR, but *DCIR* expression is detected in CD4+ T cells and CD8+ T cells of rheumatoid arthritis patients (Eklow et al. 2008). Interestingly, surface expression of DCIR is induced in CD4+ T cells not only by HIV infection but also by soluble factors from HIV-infected cells. The induction of DCIR in CD4+ T cells results in increases of HIV binding, replication, and transmission to uninfected CD4 + T cells (Lambert et al. 2010). The pathological importance of DCIR expression on DCs and CD4+ T cells in HIV pathogenesis is still obscure, but the types of DCIR-expressing cells imply potential roles of DCIR in the pathogenesis. Contribution of ITIM-dependent signaling in HIV infection has also not yet been elucidated completely, but pharmacologic inhibitors and gene silencing with oligonucleotides suggest that phosphatases (SHP-1 and SHP-2) and tyrosine kinases are involved in HIV binding to DCIR-expressing Raji cells (Lambert et al. 2011). Furthermore,

DCIR is suggested to be a potential receptor for HCV. HCV glycoprotein E2, which inhibits IFN-α and IFN-λ production, binds DCIR on pDCs from healthy donors, and treatment with anti-DCIR antibody recovers the cytokine production (Florentin et al. 2012).

7.6 DCIR Maintains Immune Homeostasis by Negatively Regulating DCs

DCIR is an important negative regulator in controlling autoimmune diseases. Mouse *Dcir* has been identified as one of potential pathological genes related to rheumatoid arthritis (Fujikado et al. 2006). The expression of *Dcir* gene was increased in the inflamed joints of two models of rheumatoid arthritis, HTLV-1-Tg mice, and *IL-1rn*–/– mice. To understand the physiological and pathological roles of DCIR, *Dcir*–/– mice have been generated. Aged *Dcir*–/– mice on the C57BL/6 background spontaneously develop several symptoms, such as swelling of joints, enthesitis, and sialadenitis (Fujikado et al. 2008). In addition, young *Dcir*–/– mice are highly susceptible to collagen-induced arthritis compared to wild-type littermates. The development of experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis, is also exacerbated in *Dcir*–/– mice, indicating a protective role for DCIR in autoimmune diseases (Seno et al. 2015). Interestingly, the proportion of CD11c+ DCs in lymph node increases in these mice and *Dcir*–/– mouse-derived BM cells differentiates more efficiently into DCs upon induction with GM-CSF compared with WT mice, suggesting that excess differentiation and expansion of DCs in *Dcir*–/– mice may cause autoimmunity. Thus, DCIR is important for the homeostasis of the immune system by, at least in part, regulating DC differentiation. Consistent with the results obtained in mouse models, polymorphisms of DCIR gene are associated with the susceptibility to rheumatoid arthritis in humans (Eklow et al. 2008; Lorentzen et al. 2007; Guo et al. 2012).

Recently, DCIR has been reported to be involved in the homeostasis of bone. DCIR deficiency increases bone volume and causes aberrant ossification in joints, and *Dcir*–/– mice spontaneously develop ankylosis accompanied by fibrocartilage proliferation and ectopic ossification. Moreover, *Dcir*–/– mice increase IFN-γ+ T cells in peripheral blood and lymph nodes. Splenic CD11c+ DCs isolated from *Dcir*–/– mice have higher potency to induce IFN-γ+ T cells, relative to WT mice. Notably, IFN-γ is identified as an osteogenic factor that promotes chondrogenesis and osteoblastogenesis (Maruhashi et al. 2015). These demonstrate that DCIR deficiency causes an increase of IFN-γ+ T cells, which accelerates mineralization of OBs and proliferation of chondrocytes, leading to the increased bone volume. Thus, DCIR has broad functional activities and is involved in not only the homeostasis of the immune system but also the bone metabolism.

DCIR-Fc protein weakly binds commensal microbiota, and *Dcir*–/– mouse-bone-marrow-derived DCs (031932-UCD) secreted higher concentrations of TNF-α in response to heat-killed microbiota. However, *Dcir* deficiency only marginally influenced the development of DSS-induced colitis, a mouse model for ulcerative colitis, and pro-inflammatory cytokine production (TNF-α, IL-6, and IL-1β) in colon organ culture, indicating a limited role of DCIR in experimental colitis (Hutter et al. 2014). On the other hand, DCIR has a protective role in the pathogenesis of chikungunya virus (CHIKV) infection (Long et al. 2013). CHIKV-induced weight loss and edema were exacerbated in *Dcir*–/– mice but not in DC-SIGN–/– and SIGNR3–/– mice. Cell infiltration and inflammatory tissue damage were enhanced in the fascia of the inoculated foot and the ankle joint of *Dcir*–/– mice. Cytokine production in response to CHIKV in DCIR-defective BMDCs was enhanced even in the absence of viral replication in DCs (Long et al. 2013). There was no evidence that DCIR directly interacts with CHIKV. Thus, DCIR plays a protective role in limiting the CHIKV-induced inflammatory response and subsequent tissue and joint damages, probably by regulating the activation state and pathological potential of the inflammatory cells entering the virally infected tissue.

Interestingly, *Dcir*–/– mice were protected from neurological symptoms of cerebral malaria (CM) during *Plasmodium berghei* infection, although the incidence of parasitemia was similar between *Dcir*–/– and wild-type mice (Maglinao et al. 2013). *Dcir* deficiency diminished infiltration of CD8+ T cell and inflammation in the brain, TNF-α level in sera, and frequency of activated T cells in the spleen. Because these phenotypes are rather unexpected from the function of DCIR in DCs and macrophages, roles of DCIR in the pathogenesis of CM remain unclear.

DCIR can be used as a target molecule on DCs for enhancing antigen presentation to T cells. Targeting of antigens through CLRs on DCs induces efficient antigen presentation to MHC-I-restricted or MHC-II-restricted T cells (Bonifaz et al. 2004; Carter et al. 2006). Similar to the other CLRs, targeting of DCIR with antigen-conjugated anti-DCIR antibody on human pDCs enhanced antigen-specific proliferation of peripheral blood leukocytes, because antigens are internalized together with DCIR after receptor triggering (Meyer-Wentrup et al. 2008). In addition, delivery of antigens by DCIR to human skin-derived DCs and blood-derived DCs induced cross-presentation to CD8+ T cells. TLR7/8 agonists or combination of TLR7/8 agonist and CD40L further augmented the DCIR-mediated cross-presentation (Klechevsky et al. 2010). Anti-DCIR antibody conjugated with antigens may become a strong inducer of cytotoxic CD8+ T cells for virus-infected cells and tumors.

7.7 Conclusion

DCIR is a unique CLR containing the ITIM in the cytoplasmic part. As *Dcir*–/– mice spontaneously develop autoimmunity and bone morphogenic abnormality, this molecule has important roles in not only the immune system but also the bone

metabolism. DCIR in DCs negatively regulates their differentiation and functions, and inhibition of DCIR shapes T cells to produce IFN-γ. DCIR is suggested to be involved in the susceptibility to HIV infection, CHIKV-induced pathogenesis, and *Plasmodium berghei*-induced cerebral malaria. In addition, targeting this molecule to deliver antigens to DCs efficiently induces antigen-specific T cell responses. Further investigation is needed to elucidate its functional ligands and the precise mechanisms to regulate the immune and bone system.

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