Glycoconj J (2012) 29:273–284
DOI 10.1007/s10719-012-9419-9

The C-type lectin-like receptors of Dectin-1 cluster in natural killer gene complex

Jianhui Xie

Received: 13 April 2012 / Revised: 5 June 2012 / Accepted: 12 June 2012 / Published online: 12 July 2012
© Springer Science+Business Media, LLC 2012

Abstract Natural killer gene complex (NKC) encodes a group of proteins with a single C-type lectin-like domain, (CTLD) which can be subdivided several subfamilies according to their structures and expression patterns. The receptors containing the conserved calcium binding sites in the CTLD fold belong to group II of C-type lectin superfamily and are expressed on myeloid cells and non- myeloid cells. The receptors lacking conserved calcium binding sites in the CTLD fold have evolved to bind ligands other than carbohydrates independently on calcium and thereby are named as C-type lectin-like receptors. The C-type lectin-like receptors are previously thought to be exclusively expressed on natural killer (NK) cells and enable NK cells to discriminate self, missing self or altered self. However, some C-type lectin-like receptors are identified in myeloid cells and are intensely investigated, recently. These myeloid C-type lectin-like receptors, especially Dectin-1 cluster, have a wide variety of ligands, including those of exogenous origin, and play important roles in the physiological functions and pathological processes including immune homeostasis, immune defenses, and immune surveillance. In this review, we summarize each member of the Dectin-1 cluster, including their structural profiles, expression patterns, signaling properties as well as known physiological functions.

Keywords C-type lectin-like receptors · Dectin-1 cluster · Natural killer gene complex · Myeloid cells

J. Xie (✉)
Key Laboratory of Glycoconjugate Research, Ministry of Health,
Department of Biochemistry and Molecular Biology,
Shanghai Medical College, Fudan University,
Shanghai 200032, China
e-mail: jxgu@shmu.edu.cn

Abbreviations

NKC	Natural killer gene complex
CTLD	C-type lectin-like domain
hemITAM	A single tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
Syk	Spleen tyrosine kinase
CLR	C-type lectin
TLR	Toll-like receptor
MHC	Major histocompatibility complex
ROS	Reactive oxygen species
PRR	Pattern recognition receptor
DC	Dendritic cell

Introduction

The region on mouse chromosome 6 was first found to contain genes encoding NKR-P1 and Ly49 in natural killer cells, which gives rise to the term natural killer gene complex (NKC) [1, 2]. A significant number of genes in NKC encode type II transmembrane proteins with a single C-type lectin-like domain (CTLD) in their extracellular region, which play important roles in immune homeostasis and surveillance [3, 4]. These receptors can roughly be subdivided into two groups according to the structural motif CTLD: classical and non-classical C-type lectins (CLR) [5]. Classical C-type lectins, such as Dectin-2 and Mincle, bear carbohydrate-recognition domains that bind glycan ligands in a calcium-dependent manner [6, 7]. In contrast, non-classical C-type lectins, such as NKG2D and CD69, share structural homology with those classical counterparts, but lack the residues involved in calcium binding in the CTLD fold, and have evolved to recognize non-sugar ligands such as proteins and lipids [8–10]. These non-classical C-type lectins are traditionally thought to be

selectively expressed on NK cells, NKT cells and subsets of T cells [3]. However, some of these receptors are recently identified to be primarily expressed on myeloid cells [11, 12]. These non-classical C-type lectins, also named as C-type lectin-like receptors, on myeloid cells appear to have a wider variety of functions and ligands, compared with their counterparts on NK cells that primarily control cellular activation through recognition of major histocompatibility complex (MHC) class I and related molecules [13, 14]. In recent years, intense investigations are focused on these myeloid C-type lectin-like receptors, especially on those of Dectin-1 cluster.

The Dectin-1 cluster consists of LOX-1, Dectin-1, CLEC-1, CLEC-2, CLEC9A, CLEC12A, and CLEC12B. The cartoon representation of their structures is shown in the order of the genomic organization in human chromosome 12 (Fig. 1). They share a common structure that contains a single extracellular CTLD, a neck region, a transmembrane region and a short cytoplasmic tail [12]. The pre-mRNA of them can be alternatively spliced to generate invariants, which may be an important way to regulate receptor functions. The potential N-linked glycosylation sites infrequently appear in the long loop region, which plays a key role in specific ligand binding for the CTLD [15], implying that the modification of N-linked glycosylation may not directly participate in the ligand binding. Most of these receptors possess cysteine residues in the neck region, which can form the intrachain disulfide bond. Dectin-1, CLEC-2 and CLEC9A bear a single tyrosine-based activation motif, termed hemITAM motif, in their cytoplasmic tails and function as the activating receptors [16]. In contrast, CLEC12A and CLEC12B bear the immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic tails and may play a role in the immune homoeostasis. Despite possessing several potential phosphorylation sites, the cytoplasmic tails of LOX-1 and CLEC-1 have a lack of any known consensus signaling motifs. These myeloid C-type lectin-like receptors selectively bind a wide range of ligands through the structurally conserved CTLD fold with some variations. The natural ligands of some members of this cluster remain to be identified and their biological functions are unclear. Here, we in brief review the members of the Dectin-1 cluster, respectively.

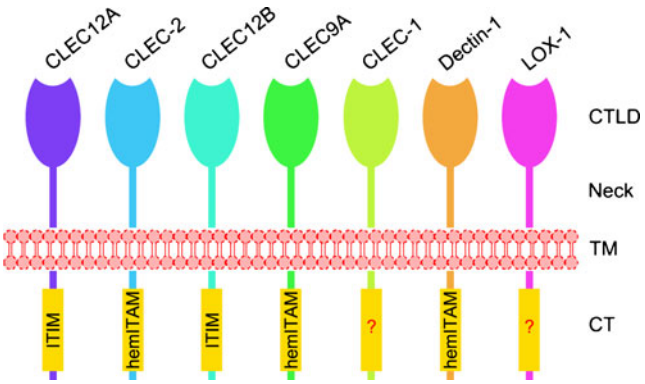

Fig. 1 Schematic model of C-type lectin-like receptors of Dectin-1 cluster. Members of Dectin-1 cluster are type II transmembrane proteins and share a common structure that contains a single extracellular C-type lectin-like domain (CTLD), a neck region (Neck), a transmembrane region (TM) and a short cytoplasmic tail (CT). In their cytoplasmic tails, Dectin-1, CLEC-2 and CLEC9A bear a single tyrosine-based activation motif (hemITAM). CLEC12A and CLEC12B bear an immunoreceptor tyrosine-based inhibitory motif (ITIM). It is not yet known whether the cytoplasmic tails of LOX-1 and CLEC-1 have signaling motifs

Myeloid C-type lectin-like receptors in Dectin-1 cluster

LOX-1 (CLEC8A)

Lectin-like oxLDL receptor (LOX-1) was originally isolated from a cDNA library of the bovine aortic endothelial cells by screening receptors for oxidized LDL (oxLDL) [10]. Mouse and rat LOX-1 have the unique triple-repeat sequences in the neck region compared with human and bovine homologs [17]. The neck region of LOX-1 possesses a cysteine residue that can form the interchain disulfide bond, resulting in the homo-dimerization of LOX-1 [18]. The modification of N-linked glycosylation on the extracellular region of LOX-1 facilitates the intracellular transport and ligand binding [19]. LOX-1 can be cleaved at the juxtamembrane region by PMSF-sensitive proteases and sheds from the cell surface [20]. Cytokines including IL-18 and TNF-α can stimulate the cleavage of LOX-1 to be the soluble LOX-1, which is found to be elevated in patients with acute coronary syndrome, coronary artery disease and type 2 diabetes [21–23]. LOX-1 is found to be expressed on vascular endothelial cells, smooth muscle cells, fibroblasts and chondrocytes [24–26]. The expression of LOX-1 is also detected in monocytes, macrophages, immature dendritic cells (DCs), platelets and B cells, but not in mature DCs and T cells [27, 28]. The level of LOX-1 expression can be stimulated by a number of inflammatory cytokines and pathologic factors, which indicates multiple roles of LOX-1 *in vivo* [24, 29, 30]. An isoform of LOX-1, named as LOXIN, lacks part of the C-terminus lectin-like domain, resulting in a reduction of plasma membrane localization and the loss of oxLDL-binding activity, which is thought to have protective effect against acute myocardial infarction [31, 32]. Co-expression of LOXIN with LOX-1 can form hetero-oligomers and has an inhibitory role in LOX-1 activation by a decrease of LOX-1 on the cell surface and a marked impairment of oxLDL binding and uptake. A functional SNP (G501C), resulting in a missense mutation (K167N), is shown to affect LOX-1-mediated ligand binding and ERK1/2 activation, which may be associated with the risk of myocardial infarction [33].

OxLDL is able to stimulate the activation of LOX-1 on endothelial cells and smooth muscle cells to induce cell dysfunction or apoptosis [34]. Mice with the deletion of LOX-1 and LDLR have a reduction in atherogenesis in association with the decreased proinflammatory and pro-oxidant signals [35]. The induction of LOX-1 expression on macrophages can increase the oxLDL uptake, which contributes to foam cell formation [36, 37]. Although LOX-1 has the ability to bind and internalize oxLDL, mice with the deletion of LOX-1 show no significant reduction in uptake of oxLDL by macrophages [38]. LOX-1 is structurally thought to be a C-type lectin-like receptor while LOX-1 is functionally classified as the type E scavenger receptor. As a multiligand receptor, LOX-1 binds various ligands, including oxLDL, aged and apoptotic cells, activated platelets, heat shock proteins, C-reactive protein as well as gram$^+$ and gram$^-$ bacteria [39, 40]. As an endocytic receptor, LOX-1 utilizes a novel cytoplasmic motif (DDL) to internalize oxLDL by a clathrin-independent and dynamin-2-dependent pathway [41]. It has been shown that LOX-1 can mediate the phagocytosis of aged/apoptotic cells by the recognition of phosphatidylserine in a Ca^{2+}-dependent manner [42, 43]. Recently, Parlato *et al*. reported that IFN-α-conditioned DCs exhibit a marked up-regulation of LOX-1 expression, which contributes the uptake of apoptotic cells and the cross-presentation of cellular antigens [44]. In fact, LOX-1 on DCs is able to recognize and take up Hsp60 and Hsp70 together with the cross-presentation of coupled antigens [28, 45]. Also, LOX-1 acts as a cell-adhesion molecule involved in leukocyte recruitment during inflammation and supporting the tether of the binding bacteria by the recognition of unidentified ligands [46–48].

Although there is a lack of any known consensus signaling motifs within its cytoplasmic tail, LOX-1 can mediate or modulate a number of downstream signaling events. The binding of oxLDL to LOX-1 on endothelial cells increases production of intracellular reactive oxygen species (ROS) to induce the activation of NF-κB and reduce the intracellular concentration of nitric oxide. The activation of LOX-1 is able to stimulate a series of signaling molecules, including protein kinase C (PKC) and ERK1/2, which subsequently regulate the expression of several genes leading to diverse cell responses [49–51]. Recently, Sugimoto *et al*. reported that the membrane type 1 matrix metalloproteinase (MT1-MMP) forms a complex with LOX-1, which is crucial for RhoA and Rac1 activation together with RhoA-dependent endothelial NO synthase protein, Rac1-mediated NADPH oxidase activity, and ROS generation [52]. In contrast, LOX-1 is involved in redox-sensitive, Akt/eNOS and Ca^{2+} signaling pathways in monocyte adhesion to endothelial cells independent of the oxLDL–LOX-1 axis [53]. However, the signaling events triggered by LOX-1 in macrophages and DCs remain little known.

Dectin-1 (CLEC7A)

Dectin-1 (DC-associated C-type lectin-1) was originally identified through subtractive cDNA cloning from XS52 DCs and independently was isolated from RAW264.7 cells using the β-glucan-rich particle zymosan [54, 55]. The neck region of Dectin-1 lacks the cysteine residues that form the interchain disulfide bond, resulting in the monomer of Dectin-1 expression on cells [56]. In contrast to the murine Dectin-1 with two probable N-glycosylation sites at CTLD, human homolog has only one predicted N-linked glycosylation site at the neck region [57]. Although the N-linked glycosylation of Dectin-1 affects the cell surface expression of the molecule, the functional significance of the glycosylation differences including position and number remains to be clarified [58]. Dectin-1 is predominantly expressed on myeloid cells, including DCs, monocytes, macrophages, and neutrophils, as well as weakly on a subset of T cells and human homolog is also expressed on B cells, eosinophils, and mast cells [59]. Surface expression of Dectin-1 can be regulated by cytokines and other agents, including microbial components [60]. Dectin-1 is alternatively spliced generating two major and a number of minor isoforms, which have different functionalities [61]. A minor human isoform (hDectin-1E) with a lack of the neck and transmembrane regions shows a cytoplasmic localization and is capable of interacting with the cytoplasmic scaffold RanBPM [62].

As a non-Toll-like receptor (TLR) pattern recognition receptor (PRR), Dectin-1 specifically recognizes β-1-3-glucans in a calcium-independent manner. Within the CTLD of Dectin-1 there is an amino acid sequence WIH, which is conserved in all identified Dectin-1 homologs. Mutational analyses have revealed that two residues (W221 and H223) that flank a shallow groove on the protein surface are required for β-glucan binding [63]. The nature of ligands determines the intracellular trafficking of Dectin-1 and Dectin-1-induced inflammatory response. With the particulate ligand, Dectin-1 traffics to lysosomes. However the receptor was observed to recycle when associated with laminarin, a soluble ligand [64]. Dectin-1 signaling is only activated by particulate β-glucans, which cluster the receptor in synapse-like structures from which regulatory tyrosine phosphatases CD45 and CD148 are excluded [65]. Large β-glucan particles that induce frustrated phagocytosis lead to enhanced downstream signaling events and production of pro-inflammatory cytokines [66, 67]. Recognition of a number of fungal species by Dectin-1 triggers protective responses including the production of ROS and the release of cytokines and is thought to play an important role in antifungal immunity [68]. Dectin-1 is also reported to be involved in mycobacterial infections and promote bacterial uptake as well as the induction of a number of cytokines and

chemokines [69, 70]. The ligand recognized by Dectin-1 remains to be identified since mycobacteria do not have β-glucans. In addition to exogenous ligands, Dectin-1 is reported to recognize an unidentified self-ligand on T cells to stimulate cell proliferation *in vitro* [54]. The endogenous ligand of Dectin-1 is also detectable on tumor cells, which could be induced by apoptosis [71]. Dectin-1-mediated recognition facilitates the uptake of apoptotic cells and cross-presentation of cellular antigens. The delivery of antigens by Dectin-1 on DCs has been shown to induce antigen-specific $CD4^+$ and $CD8^+$ T cell responses, which indicates Dectin-1 as a potential targeting molecule for immunization [72, 73].

There are two tyrosine residues within the cytoplasmic tail of Dectin-1. However only phosphorylation of the membrane proximal one (Try15) within the hemITAM sequence is required. This is sufficient to mediate signaling through Syk [74]. Upon ligand binding, this motif becomes tyrosine phosphorylated by Src family kinases and thereby recruits Syk, which initiates a subsequent signaling cascade. The receptor lacks cysteine residues in its stalk region and binding of the two SH2 domains of Syk to separate Dectin-1 molecules, which is thought to induce the dimerization of two Dectin-1 molecules. The Syk-mediated signaling induces the formation of CARD9/Bcl-10/Malt-1 scaffold, which activates both canonical NF-κB signaling and noncanonical NF-κB signaling (RelB) pathway [75–77]. The activation of Syk also triggers the activation of p38, ERK, and JNK cascades, and NFAT as well as the generation of ROS [78–81]. The binding of ligand to Dectin-1 also is reported to induce a second signaling pathway mediated by the serine-threonine kinase Raf-1 that can antagonize Syk-induced RelB activation [82]. Notably, Dectin-1-mediated signaling and cell activation is shown to be cell-type specific [83]. Collectively, The Dectin-1 activation induces both activation Th1 and Th-17 cell immune responses, thus providing antifungal immunity.

CLEC-1 (CLEC1A)

CLEC-1 was originally identified through a bio-informatic approach to screen molecules with similarity to C-type lectin-like receptors on NK cells [84]. Apart from six conserved cysteine residues, the CTLD of CLEC-1 structurally displays two more cysteines, which may form a fourth intra-chain disulfide bond. Similar to LOX-1, CLEC-1 could form disulfide-linked homodimers and two conserved cysteine residues in the stalk domain could potentially be used in the formation of disulfide bridges between protein subunits [85]. CLEC-1 mRNA can be detected in endothelial cells and some myeloid cells including monocytes, granulocytes and dendritic cells, but absent from NK cells and lymphocytes [84]. Although immunoregulatory mediators could increase CLEC-1 expression, the effect of inflammatory stimuli on CLEC-1 expression seems dependent on cell sources and subtypes [85, 86]. CLEC-1 does not reach the cell surface in endothelial or fibroblast-like cell transfectants and recently Sattler *et al.* reported that CLEC-1 is actually localized to the endoplasmic membrane compartment in human endothelial cells, which may serve as an intracellular PRR [85]. Neither TGF-β nor inflammatory stimuli could promote significant translocation of CLEC-2 to the cell surface, which supports a normal intracellular localization of CLEC-1 independent of the activation state of cells. In contrast, Thebault *et al.* reported the surface expression of CLEC-1 on rat DCs and macrophages as well as a low level on endothelial cells, implying that unknown molecules could facilitate the delivery of CLEC-1 to cell surface [86].

Although there is a conserved tyrosine residue within the cytoplasmic tail of CLEC-1, it is not found within a known consensus signaling motif. There is a positively charged arginine residue very close to the predicted transmembrane region. The arginine residue in the transmembrane region is essential for interaction with ITAM-containing adaptor molecules [87]. However, this residue did not mediate the association of CLEC-1 with the signaling adaptor FcRγ chain (unpublished data). How CLEC-1 activates the downstream signaling events remains elusive. The ligand of CLEC-1 still remains unknown to date and little is known about its physiological role. Recently, Thebault *et al.* reported that CLEC-1 could be up-regulated in a model of rat allograft tolerance, which may be induced by alloantigen-specific regulatory $CD4^+CD25^+$ T cells [86]. The increased expression of CLEC-1 is associated with a low expression of IL-17 and a high expression of Foxp3. *In vitro* inhibition of CLEC-1 expression increases the subsequent differentiation of allogeneic Th-17 T cells and decreases the regulatory $Foxp3^+$ T cells. However, how CLEC-1 signaling modulates subsequent T cell activation needs to be further clarified.

CLEC-2 (CLEC1B)

CLEC-2 is first identified as one member of non-classical C-type lectins by sequence similarity to C-type lectin-like molecules with immune functions [84]. The amino acid sequence of CLEC-2 homologs holds with an extraordinary conservation, compared with other members of Dectin-1 cluster. The functional structures including N-linked glycosylation sites and ligand-binding domain also implement a structural and functional conservation. Two potential N-linked glycosylation sites (N120 and N134) of CLEC-2 near α1 helix of the CTLD are shown to be essential for the traffic of CLEC-2 to cell surface [88]. Like LOX-1, the shedding of mouse CLEC-2 could be observed in HEK293T cell transfectants [89]. In fact, the soluble form of human CLEC-2 could be detected during platelet activation

(unpublished data). However, the functional significance of soluble CLEC-2 remains elusive. There is a conserved Cysteine residue in the neck of CLEC-2 homologs, resulting in the homodimers at least of soluble mouse CLEC-2 [89]. CLEC-2 is expressed in a number of hematopoietic lineages including neutrophils, monocytes, macrophages, dendritic cells, NK cells, granulocyte, and B cells [90, 91]. CLEC-2 is also reported to be expressed in liver sinusoidal endothelial cells and Kupffer cells [92]. In contrast, CLEC-2 is highly expressed on the surface of platelets and megakaryocytes [92]. Several alternative splicing isoforms of CLEC-2 mRNA are detected in tissues and cell lines with unknown functions [89].

By rhodocytin affinity chromatography to isolate platelet components, CLEC-2 was identified as the receptor for snake venom protein rhodocytin [93]. Analysis of the crystal structure of CLEC-2 reveals that the semi-helical loop region and flanking residues dominates the surface which is available for ligand binding [94]. Recombinant CLEC-2 expressed in bacteria is able to bind rhodocytin with the lack of glycosylation modification revealed by computational bioinformatics with N- and O- glycosylation site prediction programs, and confirmed by SDS-PAGE and mass spectroscopy, which suggests that the recognition of rhodocytin by CLEC-2 is based on protein-protein interaction. In fact, both potential N-linked glycosylation sites of CLEC-2 are located on the bottom of the CTLD fold, which might not be involved in ligand binding, directly. Recently, podoplanin, a type-I transmembrane mucin-like glycoprotein, was identified as the endogenous ligand of CLEC-2 [95]. The association of podoplanin with CLEC-2 is able to induce platelet activation and aggregation. The binding of podoplanin to CLEC-2 requires sialic acid on O-glycans of podoplanin. Although a disialyl-core1-attached glycopeptide containing the platelet aggregation stimulating (PLAG) region is enough for the recognition of CLEC-2, the stereostructure of the podoplanin protein seems critical for the CLEC-2-binding activity of podoplanin [96]. Since CLEC-2 possesses a CTLD with a lack of the consensus sequence for binding calcium and sugars, the precise mechanism of the binding of sialic acid to CLEC-2 needs to be further clarified.

CLEC-2 possesses a hemITAM motif in its cytoplasmic tail and the tandem SH2 domains of Syk are able to bind this phosphorylated motif [93]. Unlike the ITAM motif of the GPVI/FcRγ chain, this motif is mainly phosphorylated by Syk itself upon binding of rhodocytin, which is dependent on the translocation of CLEC-2 to lipid rafts and actin polymerization together with secondary mediators and Rac1 [97, 98]. Subsequent activation of Syk results in multiple downstream signaling events including tyrosine phosphorylation of LAT, SLP-76, and Vav1/3 as well as activation of Btk, Rac1, PI3-kinase, and phospholipase Cγ2 (PLCγ2) [93, 99]. The ITIM-containing receptors on platelets such as PECAM-1 and G6B-b have an inhibitory action on CLEC-2-mediated signaling by recruitment of the tandem SH2 domain-containing tyrosine phosphatases such as SHP-1 and SHP-2 [100, 101].

Podoplanin is expressed in lymphatic endothelial cells, type I alveolar cells, and kidney podocytes, but not in vascular endothelial cells [102]. Recent reports indicate that the interaction of podoplanin and CLEC-2 is necessary for lymph/blood vessel separation during embryonic development [103, 104]. The increased expression of podoplanin during malignant transformation is observed on cells derived from several tissues [105]. The association of podoplanin and CLEC-2 triggers the platelet activation which has a crucial role in the progression of tumor. The recognition of podoplanin by CLEC-2 on platelets is also reported to facilitate HIV-1 dissemination [92, 106]. Virion incorporation of podoplanin from HEK293T cells appears to be required for efficient CLEC-2-dependent HIV-1 interaction. In contrast, PBMC-derived viruses require unidentified factors other than podoplanin for CLEC-2-dependent capture. In contrast, less is known about the role of CLEC-2 in myeloid cells. Kerrigan *et al*. reported that murine CLEC-2 is expressed in peripheral blood neutrophils and can mediate phagocytosis of antibody-coated beads. Stimulation of rhodocytin on neutrophils induces the production of proinflammatory cytokines, including TNF-α [90]. However, results from chimeric analyses reveal that signaling mediated by CLEC-2 does not induce the respiratory burst in a macrophage cell line [90]. Using a monoclonal antibody, Mourao-Sa *et al*. reported a more wide expression of CLEC-2 on leukocytes. Antibody-mediated ligation of CLEC-2 initiates hemITAM-dependent signaling *via* Syk, Ca^{2+} and NFAT, which modulates TLR signaling to selectively promote production of IL-10 *in vitro* and *in vivo*, but not sufficiently activate NF-κB pathway and fail to stimulate cytokine production by DCs [91]. In fact, both NF-κB and NFAT activation can be observed in Dectin-1 signaling. The precise signaling mediated by CLEC-2 in myeloid cells awaits the investigation using physiologic ligands.

CLEC9A

CLEC9A (also named as DC, NK lectin group receptor-1 (DNGR-1)) is a very recently characterized receptor and shares a ~20% identity with other members of Dectin-1 cluster [107–109]. The stalk region of human CLEC9A has two cysteine residues, potentially involved in receptor dimerization, and one predicted N-linked glycosylation site, which is modified by carbohydrate. In contrast, the mouse homolog lacks a cysteine residue in the neck region and has two more predicted N-linked glycosylation sites at the extracellular domain. Human CLEC9A has a high level of expression in thymus, spleen and brain as well as a moderate

level in liver, lung and testis, revealed by RT-PCR analysis [107, 109]. In peripheral blood, the expression of human CLEC9A appears restricted to BDCA3$^+$ DCs although this receptor was also detected on small subsets of monocytes. In mouse, CLEC9A is expressed at high levels on CD8α^+ DCs, which are the putative equivalent of human BDCA3$^+$ cells, and at low levels on plasmacytoid DCs [108]. The selective expression of CLEC9A on CD8α^+-like DCs together with its endocytic capacity make it an attractive target for antigen delivery to DCs. In fact, antibody-coupled antigens are able to target CLEC9A to allow the efficient cross-presentaion to CD8$^+$ T cells by CD8α^+ DCs [109]. In mouse model, the delivery of antigens to MHC class I pathway by CLEC9A elicits robust antitumor responses with co-administration of the antibody against CD40 [108]. Also, the delivery of antigens mediated by CLEC9A can be efficiently presented on MHC class II molecules, resulting in potent CD4$^+$ T cell responses and humoral immunity [110, 111]. The adjuvants including poly I:C is able to potentiate the responses elicited by CLEC9A. Therefore, CLEC9A is thought to be a promising target for antigen delivery to enhance the efficiency of vaccines.

CLEC9A bears a hemITAM sequence within its cytoplasmic tail, which might act as an activation motif. A chimera with the replacement of extracellular domain of CLEC9A by that of Dectin-1 could signal through this motif to recruit Syk kinase and induce the production of pro-inflammatory cytokines on ligand binding [107]. Similarly, antibody-mediated crosslinking of CLEC9A induced phosphorylation of endogenous Syk in a CLEC9A-transfected B cell line [112]. However, antibody-mediated crosslinking of CLEC9A does not induce or regulate the maturation of DCs in the absence or presence of TLR stimuli [109, 113]. Recently, Sancho *et al*. reported that CLEC9A recognizes an endogenous unidentified ligand, which is hidden in cells viable or undergoing primary apoptosis, but exposed when cells undergo primary or secondary necrosis [112]. In contrat, the ligand of CLEC9A exposed on necrotic corpses is able to trigger hemITAM-dependent CLEC9A signaling *via* Syk in CD8α^+ DCs. More recently, CLEC9A is shown to bind to damaged cells by the recognition of F-actin [114, 115]. F-actin is able to trigger CLEC9A signaling *via* Syk, which might be important for CLEC9A-dependent cross-presentation. However, the stimulation of F-actin did not lead to DC maturation and cytokine responses, implying that CLEC9A is not involved in immune regulation [114]. It is interesting to clarify the difference between CLEC9A signaling and other hemITAM-containing CLR signaling.

CLEC12A

CLEC12A (also named as C-type lectin-like molecule-1 (CLL-1), Killer cell lectin-like receptor 1 (KLRL1), Myeloid Inhibitory C-type Lectin-like Receptor (MICL) or dendritic cell associated C-type lectin 2 (DCAL-2)) was independently cloned by several groups [116–118]. The extracellular portion of human CLEC12A comprises six potential N-glycosylation sites with the two more C-terminal sites of the stalk region contributing the majority of cell-specific N-glycosylation [118, 119]. This feature is thought to prevent its dimerization despite the presence of two conserved cysteine residues. In contrast, mouse homolog has one less stalk N-glycosylation site and has been reported to exist as a dimer [120]. The mRNA of human CLEC12A has a high level of expression in peripheral blood leukocytes (PBMCs) and bone marrow as well as a moderate level in lung. The surface expression of human CLEC12A has been detected on several hematopoietic lineages, including monocytes, DCs, granulocytes, and B cells at a low level, but has been shown to be negative on blood NK and T cells. The mouse orthologue has been found to be expressed on monocytes, macrophages, DCs, neutrophils, eosinophils, and basophils, B cells, as well as on bone marrow NK cells, but absent from blood NK cells [120, 121]. CLEC12A could be alternatively spliced, generating at least three variants including isoforms α (full-length form), β (with a lack of the exon encoding the transmembrane region), and γ (with the second intron unspliced, resulting in the introduction of a stop codon) [118].

A chimera of CLEC12A with the replacement of the CTLD and partial stalk by that of Dectin-1 is able to recognize, but not internalize, zymosan, which reveals the inability of uptake for the cytoplasmic tail of CLEC12A [118]. The cytoplasmic tail of CLEC12A possesses an ITIM sequence, which is able to recruit the tyrosine phosphatases SHP-1 and SHP-2, but not SHIP [117]. As an inhibitory receptor, this chimera suppresses Dectin-1-mediatd the release of TNF-α in response to zymosan [118]. In fact, the expression of CLEC12A is down-regulated following myeloid cell activation, which may attenuate negative regulation to promote cellular activation [119].

In immature human DCs, Cross-linking of CLEC12A alone could induce phosphorylation of both p38 and ERK1/2, up-regulation of CCR7 expression, and the release of cytokines including IL-6 and IL-10, but not induce DC maturation [122]. In the presence of TLR agonists, CLEC12A signals suppress TLR-induced IL-12 expression and the induction of Th1 cells. In contrast, CLEC12A ligation enhances CD40L-induced IL-12 production and Th1 polarization. Mouse CLEC12A is highly expressed on splenic CD8$^+$ DCs and plasmacytoid DCs [121]. A proportion of splenic CD8$^-$ DCs with CLEC12A, which robustly produce cytokines including IL-12, as well as efficiently induced Th1 responses in response to CpG stimulation, defines a distinct CD8α^- DC subset [123]. Antibody-mediated cross-linking of CLEC12A has no effects on

mouse DC activation [121]. Targeting antigens to mouse CLEC12A on DCs by a rat mAb elicits robust anti-rat Ig responses and promote antigen-specific T cell proliferation in conjunction with TLR stimuli [121].

Apart from being thought as a regulator of immune homeostasis, CLEC12A has a pathological role. In acute myeloid leukemia (AML), there is a high level of CLEC12A expression with the majority of AML cases (68 of 74) [116]. Importantly, CLEC12A is also found to be present on the $CD34^{+}CD38^{-}$ stem cell compartment. $CD34^{+}CLEC12A^{+}$ population is able to engraft to generate CLEC12A-positve blasts in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice [124]. The expression of CLEC12A on $CD34^{+}CD38^{-}$ cells is associated with clinical remission or relapse status, which enables CLEC12A as AML-specific stem-cell marker and possibly antigen-targeting in future. Additionally, an increased gene expression of CLEC12A correlates with rheumatoid factor (RF) values in rheumatoid fibroblast-like synovial cells. Using a phenotype-based approach, a potential genetic association of CLEC12A with rheumatoid arthritis is observed [125, 126].

It is required to identify the ligand of CLEC12A to clearly understand the physiological and pathological role of CLEC12A. Pyz *et al*. report that CLEC12A recognizes an endogenous ligand in a variety of murine tissues including bone marrow, thymus, heart, spleen and kidney, suggesting that the receptor has a role in the maintenance of immune homeostasis [120]. Recently, Zhang *et al*. identified a panel of peptides with a conserved LRS/T sequence that could specifically bind to CLEC12A *via* the phage display [127]. However, the ligand of CLEC12A is to date unknown.

CLEC12B

CLEC12B is originally identified during a database search for sequences with homology to NKG2D and shows a high similarity to CLEC12A (34% similarity) [128]. Although the CTLD of CLEC12B shows highest homology to human NKG2D (36% similarity), CLEC12B does not bind NK2GD ligands tested and its ligand yet remains unknown. Human CLEC12B is widely expressed in various human tissues, except brain, and at least two isoforms generated by alternative splicing are found. One of splicing variants lacks part of C-type lectin-like domain and is thought to be nonfunctional with preferential expression in lung, mammary gland and ovary. Recently, Sattler *et al*. reported that four splicing variants were detected in several cell lines tested, which include three putative nonfunctional isoforms [129]. These truncated variants constitute the majority of transcripts in most cell types. However the full-length variant shows a low expression level, which is consistent with no detection of CLEC12B on freshly isolated monocytes or any other leukocyte population by a specific antibody against CLEC12B. The expression of CLEC12B on cell surface could be induced upon the differentiation of monocytes into macrophages and the PMA stimulation of U937 cells. In DCs, CLEC12B expression is not significantly down-regulated during DC maturation, a feature common to other C-type lectin-like receptors of the myeloid subfamily. Murine CLEC12B displays a limited expression in the testes and lung [130]. A specific antibody against murine CLEC12B reveals the expression on spermatogonial cells within the seminiferous tubules. Nevertheless, Knockout of CLEC-12B shows no obvious phenotype with regard to reproductive ability. Structurally, CLEC12B contains an ITIM sequence within its cytoplasmic tail that can recruit the inhibitory phosphatases SHP-1 and SHP-2, resulting in an inhibition of cellular activation in a transfected-cell model. The functional significance of CLEC12B needs to be further understood.

Concluding remarks

Although extensive investigations are focused on C-type lectin-like receptors in Dectin-1 cluster of natural killer gene complex, their physiological roles remain most unknown. Several members are still orphan receptors. The identification of their endogenous and/or exogenous ligands will be an important step forward for access to understanding their functional significance. Another approach to shed light on their functions will come from the characterization of their employed signal transduction pathway, especially for those without any known consensus signaling motifs. The generation of mouse knockout models will allow further unveiling their physiological functions. Collectively, the future studies will provide more insight into this cluster of C-type lectin-like receptors on myeloid cells.

Acknowledgements We thank National Basic Research Program of China (973 Program 2012CB8221004) and the National Natural Science Fund (31010103906) for financial support. The authors thank Dr. Songwen Zhang for critical reading of the manuscript.

References

1. Yokoyama, W.M., Kehn, P.J., Cohen, D.I., Shevach, E.M.: Chromosomal location of the Ly-49 (A1, YE1/48) multigene family. Genetic association with the NK 1.1 antigen. J. Immunol. **145**(7), 2353–2358 (1990)
2. Yokoyama, W.M., Ryan, J.C., Hunter, J.J., Smith, H.R., Stark, M., Seaman, W.E.: CDNA cloning of mouse NKR-P1 and genetic linkage with LY-49. Identification of a natural killer cell gene complex on mouse chromosome 6. J. Immunol. **147**(9), 3229–3236 (1991)
3. Yokoyama, W.M., Plougastel, B.F.: Immune functions encoded by the natural killer gene complex. Nat. Rev. Immunol. **3**(4), 304–316 (2003)

4. Kanazawa, N.: Dendritic cell immunoreceptors: C-type lectin receptors for pattern-recognition and signaling on antigen-presenting cells. J. Dermatol. Sci. **45**(2), 77–86 (2007)
5. Zelensky, A.N., Gready, J.E.: The C-type lectin-like domain superfamily. FEBS J. **272**(24), 6179–6217 (2005)
6. Graham, L.M., Brown, G.D.: The Dectin-2 family of C-type lectins in immunity and homeostasis. Cytokine **48**(1–2), 148–155 (2009)
7. Miyake, Y., Ishikawa, E., Ishikawa, T., Yamasaki, S.: Self and nonself recognition through C-type lectin receptor, Mincle. Self Nonself **1**(4), 310–313 (2010)
8. Biassoni, R., Cantoni, C., Pende, D., Sivori, S., Parolini, S., Vitale, M., Bottino, C., Moretta, A.: Human natural killer cell receptors and co-receptors. Immunol. Rev. **181**, 203–214 (2001)
9. Sancho, D., Gomez, M., Sanchez-Madrid, F.: CD69 is an immunoregulatory molecule induced following activation. Trends Immunol. **26**(3), 136–140 (2005)
10. Sawamura, T., Kume, N., Aoyama, T., Moriwaki, H., Hoshikawa, H., Aiba, Y., Tanaka, T., Miwa, S., Katsura, Y., Kita, T., Masaki, T.: An endothelial receptor for oxidized low-density lipoprotein. Nature **386**(6620), 73–77 (1997)
11. Hofer, E., Sobanov, Y., Brostjan, C., Lehrach, H., Duchler, M.: The centromeric part of the human natural killer (NK) receptor complex: lectin-like receptor genes expressed in NK, dendritic and endothelial cells. Immunol. Rev. **181**, 5–19 (2001)
12. Pyz, E., Marshall, A.S., Gordon, S., Brown, G.D.: C-type lectin-like receptors on myeloid cells. Ann. Med. **38**(4), 242–251 (2006)
13. Geijtenbeek, T.B., van Vliet, S.J., Engering, A., t Hart, B.A., van Kooyk, Y.: Self- and nonself-recognition by C-type lectins on dendritic cells. Annu. Rev. Immunol. **22**, 33–54 (2004)
14. Robinson, M.J., Sancho, D., Slack, E.C., LeibundGut-Landmann, S., Reis e Sousa, C.: Myeloid C-type lectins in innate immunity. Nat. Immunol. **7**(12), 1258–1265 (2006)
15. Zelensky, A.N., Gready, J.E.: Comparative analysis of structural properties of the C-type-lectin-like domain (CTLD). Proteins **52** (3), 466–477 (2003)
16. Sancho, D., Reis, E.S.C.: Signaling by myeloid C-type lectin receptors in immunity and homeostasis. Annu. Rev. Immunol. **30**, 491–529 (2011)
17. Nagase, M., Hirose, S., Fujita, T.: Unique repetitive sequence and unexpected regulation of expression of rat endothelial receptor for oxidized low-density lipoprotein (LOX-1). Biochem. J. **330** (Pt 3), 1417–1422 (1998)
18. Matsunaga, S., Xie, Q., Kumano, M., Niimi, S., Sekizawa, K., Sakakibara, Y., Komba, S., Machida, S.: Lectin-like oxidized low-density lipoprotein receptor (LOX-1) functions as an oligomer and oligomerization is dependent on receptor density. Exp. Cell. Res. **313**(6), 1203–1214 (2007)
19. Kataoka, H., Kume, N., Miyamoto, S., Minami, M., Murase, T., Sawamura, T., Masaki, T., Hashimoto, N., Kita, T.: Biosynthesis and post-translational processing of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1). N-linked glycosylation affects cell-surface expression and ligand binding. J. Biol. Chem. **275(9), 6573–6579 (2000)**
20. Murase, T., Kume, N., Kataoka, H., Minami, M., Sawamura, T., Masaki, T., Kita, T.: Identification of soluble forms of lectin-like oxidized LDL receptor-1. Arterioscler. Thromb. Vasc. Biol. **20** (3), 715–720 (2000)
21. Mitsuoka, H., Kume, N., Hayashida, K., Inui-Hayashiada, A., Aramaki, Y., Toyohara, M., Jinnai, T., Nishi, E., Kita, T.: Interleukin 18 stimulates release of soluble lectin-like oxidized LDL receptor-1 (sLOX-1). Atherosclerosis **202**(1), 176–182 (2009)
22. Hayashida, K., Kume, N., Murase, T., Minami, M., Nakagawa, D., Inada, T., Tanaka, M., Ueda, A., Kominami, G., Kambara, H., Kimura, T., Kita, T.: Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. Circulation **112(6), 812–818 (2005)**
23. Tan, K.C., Shiu, S.W., Wong, Y., Leng, L., Bucala, R.: Soluble lectin-like oxidized low density lipoprotein receptor-1 in type 2 diabetes mellitus. J. Lipid Res. **49**(7), 1438–1444 (2008)
24. Kume, N., Murase, T., Moriwaki, H., Aoyama, T., Sawamura, T., Masaki, T., Kita, T.: Inducible expression of lectin-like oxidized LDL receptor-1 in vascular endothelial cells. Circ. Res. **83**(3), 322–327 (1998)
25. Draude, G., Hrboticky, N., Lorenz, R.L.: The expression of the lectin-like oxidized low-density lipoprotein receptor (LOX-1) on human vascular smooth muscle cells and monocytes and its down-regulation by lovastatin. Biochem. Pharmacol. **57**(4), 383–386 (1999)
26. Akagi, M., Ueda, A., Teramura, T., Kanata, S., Sawamura, T., Hamanishi, C.: Oxidized LDL binding to LOX-1 enhances MCP-1 expression in cultured human articular chondrocytes. Osteoarthr. Cartil. **17**(2), 271–275 (2009)
27. Yoshida, H., Kondratenko, N., Green, S., Steinberg, D., Quehenberger, O.: Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. Biochem. J. **334**(Pt 1), 9–13 (1998)
28. Delneste, Y., Magistrelli, G., Gauchat, J., Haeuw, J., Aubry, J., Nakamura, K., Kawakami-Honda, N., Goetsch, L., Sawamura, T., Bonnefoy, J., Jeannin, P.: Involvement of LOX-1 in dendritic cell-mediated antigen cross-presentation. Immunity **17**(3), 353–362 (2002)
29. Aoyama, T., Fujiwara, H., Masaki, T., Sawamura, T.: Induction of lectin-like oxidized LDL receptor by oxidized LDL and lysophosphatidylcholine in cultured endothelial cells. J. Mol. Cell. Cardiol. **31**(12), 2101–2114 (1999)
30. Morawietz, H., Rueckschloss, U., Niemann, B., Duerrschmidt, N., Galle, J., Hakim, K., Zerkowski, H.R., Sawamura, T., Holtz, J.: Angiotensin II induces LOX-1, the human endothelial receptor for oxidized low-density lipoprotein. Circulation **100**(9), 899–902 (1999)
31. Biocca, S., Filesi, I., Mango, R., Maggiore, L., Baldini, F., Vecchione, L., Viola, A., Citro, G., Federici, G., Romeo, F., Novelli, G.: The splice variant LOXIN inhibits LOX-1 receptor function through hetero-oligomerization. J. Mol. Cell. Cardiol. **44**(3), 561–570 (2008)
32. Mango, R., Biocca, S., del Vecchio, F., Clementi, F., Sangiuolo, F., Amati, F., Filareto, A., Grelli, S., Spitalieri, P., Filesi, I., Favalli, C., Lauro, R., Mehta, J.L., Romeo, F., Novelli, G.: *In vivo* and *in vitro* studies support that a new splicing isoform of OLR1 gene is protective against acute myocardial infarction. Circ. Res. **97**(2), 152–158 (2005)
33. Biocca, S., Falconi, M., Filesi, I., Baldini, F., Vecchione, L., Mango, R., Romeo, F., Federici, G., Desideri, A., Novelli, G.: Functional analysis and molecular dynamics simulation of LOX-1 K167N polymorphism reveal alteration of receptor activity. PLoS One **4**(2), e4648 (2009)
34. Ogura, S., Kakino, A., Sato, Y., Fujita, Y., Iwamoto, S., Otsui, K., Yoshimoto, R., Sawamura, T.: Lox-1: the multifunctional receptor underlying cardiovascular dysfunction. Circ. J. **73**(11), 1993–1999 (2009)
35. Mehta, J.L., Sanada, N., Hu, C.P., Chen, J., Dandapat, A., Sugawara, F., Satoh, H., Inoue, K., Kawase, Y., Jishage, K., Suzuki, H., Takeya, M., Schnackenberg, L., Beger, R., Hermonat, P.L., Thomas, M., Sawamura, T.: Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. Circ. Res. **100**(11), 1634–1642 (2007)
36. Li, L., Sawamura, T., Renier, G.: Glucose enhances human macrophage LOX-1 expression: role for LOX-1 in glucose-induced macrophage foam cell formation. Circ. Res. **94**(7), 892–901 (2004)

37. Smirnova, I.V., Kajstura, M., Sawamura, T., Goligorsky, M.S.: Asymmetric dimethylarginine upregulates LOX-1 in activated macrophages: role in foam cell formation. Am. J. Physiol. Heart Circ. Physiol. **287**(2), H782–H790 (2004)
38. Schaeffer, D.F., Riazy, M., Parhar, K.S., Chen, J.H., Duronio, V., Sawamura, T., Steinbrecher, U.P.: LOX-1 augments oxLDL uptake by lysoPC-stimulated murine macrophages but is not required for oxLDL clearance from plasma. J. Lipid Res. **50**(8), 1676–1684 (2009)
39. Moriwaki, H., Kume, N., Sawamura, T., Aoyama, T., Hoshikawa, H., Ochi, H., Nishi, E., Masaki, T., Kita, T.: Ligand specificity of LOX-1, a novel endothelial receptor for oxidized low density lipoprotein. Arterioscler. Thromb. Vasc. Biol. **18**(10), 1541–1547 (1998)
40. Yoshimoto, R., Fujita, Y., Kakino, A., Iwamoto, S., Takaya, T., Sawamura, T.: The discovery of LOX-1, its ligands and clinical significance. Cardiovasc. Drugs Ther. **25**(5), 379–391 (2011)
41. Murphy, J.E., Vohra, R.S., Dunn, S., Holloway, Z.G., Monaco, A.P., Homer-Vanniasinkam, S., Walker, J.H., Ponnambalam, S.: Oxidised LDL internalisation by the LOX-1 scavenger receptor is dependent on a novel cytoplasmic motif and is regulated by dynamin-2. J. Cell Sci. **121**(Pt 13), 2136–2147 (2008)
42. Oka, K., Sawamura, T., Kikuta, K., Itokawa, S., Kume, N., Kita, T., Masaki, T.: Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. Proc. Natl. Acad. Sci. U. S. A. **95**(16), 9535–9540 (1998)
43. Murphy, J.E., Tacon, D., Tedbury, P.R., Hadden, J.M., Knowling, S., Sawamura, T., Peckham, M., Phillips, S.E., Walker, J.H., Ponnambalam, S.: LOX-1 scavenger receptor mediates calcium-dependent recognition of phosphatidylserine and apoptotic cells. Biochem. J. **393**(Pt 1), 107–115 (2006)
44. Parlato, S., Romagnoli, G., Spadaro, F., Canini, I., Sirabella, P., Borghi, P., Ramoni, C., Filesi, I., Biocca, S., Gabriele, L., Belardelli, F.: LOX-1 as a natural IFN-alpha-mediated signal for apoptotic cell uptake and antigen presentation in dendritic cells. Blood **115**(8), 1554–1563 (2010)
45. Xie, J., Zhu, H., Guo, L., Ruan, Y., Wang, L., Sun, L., Zhou, L., Wu, W., Yun, X., Shen, A., Gu, J.: Lectin-like oxidized low-density lipoprotein receptor-1 delivers heat shock protein 60-fused antigen into the MHC class I presentation pathway. J. Immunol. **185**(4), 2306–2313 (2010)
46. Honjo, M., Nakamura, K., Yamashiro, K., Kiryu, J., Tanihara, H., McEvoy, L.M., Honda, Y., Butcher, E.C., Masaki, T., Sawamura, T.: Lectin-like oxidized LDL receptor-1 is a cell-adhesion molecule involved in endotoxin-induced inflammation. Proc. Natl. Acad. Sci. U. S. A. **100**(3), 1274–1279 (2003)
47. Shimaoka, T., Kume, N., Minami, M., Hayashida, K., Sawamura, T., Kita, T., Yonehara, S.: LOX-1 supports adhesion of gram-positive and gram-negative bacteria. J. Immunol. **166**(8), 5108–5114 (2001)
48. Campbell, L.A., Puolakkainen, M., Lee, A., Rosenfeld, M.E., Garrigues, H.J., Kuo, C.C.: Chlamydia pneumoniae binds to the lectin-like oxidized LDL receptor for infection of endothelial cells. Microb. Infect./Institut. Pasteur. **14**(1), 43–49 (2012)
49. Li, D., Liu, L., Chen, H., Sawamura, T., Mehta, J.L.: LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells. Arterioscler. Thromb. Vasc. Biol. **23**(5), 816–821 (2003)
50. Li, D., Mehta, J.L.: Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. Circulation **101**(25), 2889–2895 (2000)
51. Li, D., Mehta, J.L.: Intracellular signaling of LOX-1 in endothelial cell apoptosis. Circ. Res. **104**(5), 566–568 (2009)
52. Sugimoto, K., Ishibashi, T., Sawamura, T., Inoue, N., Kamioka, M., Uekita, H., Ohkawara, H., Sakamoto, T., Sakamoto, N., Okamoto, Y., Takuwa, Y., Kakino, A., Fujita, Y., Tanaka, T., Teramoto, T., Maruyama, Y., Takeishi, Y.: LOX-1-MT1-MMP axis is crucial for RhoA and Rac1 activation induced by oxidized low-density lipoprotein in endothelial cells. Cardiovasc. Res. **84**(1), 127–136 (2009)
53. Sakamoto, N., Ishibashi, T., Sugimoto, K., Sawamura, T., Sakamoto, T., Inoue, N., Saitoh, S.I., Kamioka, M., Uekita, H., Ohkawara, H., Suzuki, K., Teramoto, T., Maruyama, Y., Takeishi, Y.: Role of LOX-1 in monocyte adhesion-triggered redox, Akt/eNOS and Ca(2+) signaling pathways in endothelial cells. J. Cell. Physiol. **220**(3), 706–715 (2009)
54. Ariizumi, K., Shen, G.L., Shikano, S., Xu, S., Ritter 3rd, R., Kumamoto, T., Edelbaum, D., Morita, A., Bergstresser, P.R., Takashima, A.: Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. J. Biol. Chem. **275**(26), 20157–20167 (2000)
55. Brown, G.D., Gordon, S.: Immune recognition. A new receptor for beta-glucans. Nature **413**(6851), 36–37 (2001)
56. Herre, J., Gordon, S., Brown, G.D.: Dectin-1 and its role in the recognition of beta-glucans by macrophages. Mol. Immunol. **40**(12), 869–876 (2004)
57. Brown, G.D.: Dectin-1: a signalling non-TLR pattern-recognition receptor. Nat. Rev. Immunol. **6**(1), 33–43 (2006)
58. Kato, Y., Adachi, Y., Ohno, N.: Contribution of N-linked oligosaccharides to the expression and functions of beta-glucan receptor, Dectin-1. Biol. Pharm. Bull. **29**(8), 1580–1586 (2006)
59. Willment, J.A., Marshall, A.S., Reid, D.M., Williams, D.L., Wong, S.Y., Gordon, S., Brown, G.D.: The human beta-glucan receptor is widely expressed and functionally equivalent to murine Dectin-1 on primary cells. Eur. J. Immunol. **35**(5), 1539–1547 (2005)
60. Willment, J.A., Lin, H.H., Reid, D.M., Taylor, P.R., Williams, D.L., Wong, S.Y., Gordon, S., Brown, G.D.: Dectin-1 expression and function are enhanced on alternatively activated and GM-CSF-treated macrophages and are negatively regulated by IL-10, dexamethasone, and lipopolysaccharide. J. Immunol. **171**(9), 4569–4573 (2003)
61. Willment, J.A., Gordon, S., Brown, G.D.: Characterization of the human beta -glucan receptor and its alternatively spliced isoforms. J. Biol. Chem. **276**(47), 43818–43823 (2001)
62. Xie, J., Sun, M., Guo, L., Liu, W., Jiang, J., Chen, X., Zhou, L., Gu, J.: Human Dectin-1 isoform E is a cytoplasmic protein and interacts with RanBPM. Biochem. Biophys. Res. Commun. **347**(4), 1067–1073 (2006)
63. Adachi, Y., Ishii, T., Ikeda, Y., Hoshino, A., Tamura, H., Aketagawa, J., Tanaka, S., Ohno, N.: Characterization of beta-glucan recognition site on C-type lectin, dectin 1. Infect. Immun. **72**(7), 4159–4171 (2004)
64. Herre, J., Marshall, A.S., Caron, E., Edwards, A.D., Williams, D.L., Schweighoffer, E., Tybulewicz, V., Reis e Sousa, C., Gordon, S., Brown, G.D.: Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. Blood **104**(13), 4038–4045 (2004)
65. Goodridge, H.S., Reyes, C.N., Becker, C.A., Katsumoto, T.R., Ma, J., Wolf, A.J., Bose, N., Chan, A.S., Magee, A.S., Danielson, M.E., Weiss, A., Vasilakos, J.P., Underhill, D.M.: Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'. Nature **472**(7344), 471–475 (2011)
66. Rosas, M., Liddiard, K., Kimberg, M., Faro-Trindade, I., McDonald, J.U., Williams, D.L., Brown, G.D., Taylor, P.R.: The induction of inflammation by dectin-1 *in vivo* is dependent on myeloid cell programming and the progression of phagocytosis. J. Immunol. **181**(5), 3549–3557 (2008)
67. Hernanz-Falcon, P., Joffre, O., Williams, D.L., Reis e Sousa, C.: Internalization of Dectin-1 terminates induction of inflammatory responses. Eur. J. Immunol. **39**(2), 507–513 (2009)
68. Drummond, R.A., Brown, G.D.: The role of Dectin-1 in the host defence against fungal infections. Curr. Opin. Microbiol. **14**(4), 392–399 (2011)

69. Rothfuchs, A.G., Bafica, A., Feng, C.G., Egen, J.G., Williams, D.L., Brown, G.D., Sher, A.: Dectin-1 interaction with mycobacterium tuberculosis leads to enhanced IL-12p40 production by splenic dendritic cells. J. Immunol. **179**(6), 3463–3471 (2007)
70. Yadav, M., Schorey, J.S.: The beta-glucan receptor dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. Blood **108**(9), 3168–3175 (2006)
71. Weck, M.M., Appel, S., Werth, D., Sinzger, C., Bringmann, A., Grunebach, F., Brossart, P.: hDectin-1 is involved in uptake and cross-presentation of cellular antigens. Blood **111**(8), 4264–4272 (2008)
72. Carter, R.W., Thompson, C., Reid, D.M., Wong, S.Y., Tough, D.F.: Preferential induction of CD4+ T cell responses through *in vivo* targeting of antigen to dendritic cell-associated C-type lectin-1. J. Immunol. **177**(4), 2276–2284 (2006)
73. Ni, L., Gayet, I., Zurawski, S., Duluc, D., Flamar, A.L., Li, X.H., O'Bar, A., Clayton, S., Palucka, A.K., Zurawski, G., Banchereau, J., Oh, S.: Concomitant activation and antigen uptake via human dectin-1 results in potent antigen-specific CD8+ T cell responses. J. Immunol. **185**(6), 3504–3513 (2010)
74. Rogers, N.C., Slack, E.C., Edwards, A.D., Nolte, M.A., Schulz, O., Schweighoffer, E., Williams, D.L., Gordon, S., Tybulewicz, V.L., Brown, G.D., Reis e Sousa, C.: Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. Immunity **22**(4), 507–517 (2005)
75. Gross, O., Gewies, A., Finger, K., Schafer, M., Sparwasser, T., Peschel, C., Forster, I., Ruland, J.: Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature **442** (7103), 651–656 (2006)
76. Gringhuis, S.I., Wevers, B.A., Kaptein, T.M., van Capel, T.M., Theelen, B., Boekhout, T., de Jong, E.C., Geijtenbeek, T.B.: Selective C-Rel activation via Malt1 controls anti-fungal T(H)-17 immunity by dectin-1 and dectin-2. PLoS Pathog. **7**(1), e1001259 (2011)
77. Drummond, R.A., Saijo, S., Iwakura, Y., Brown, G.D.: The role of Syk/CARD9 coupled C-type lectins in antifungal immunity. Eur. J. Immunol. **41**(2), 276–281 (2011)
78. Leibundgut-Landmann, S., Gross, O., Robinson, M.J., Osorio, F., Slack, E.C., Tsoni, S.V., Schweighoffer, E., Tybulewicz, V., Brown, G.D., Ruland, J., Reis, E.S.C.: Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat. Immunol. **8**(6), 630–638 (2007)
79. Slack, E.C., Robinson, M.J., Hernanz-Falcon, P., Brown, G.D., Williams, D.L., Schweighoffer, E., Tybulewicz, V.L., Reis, E.S.C.: Syk-dependent ERK activation regulates IL-2 and IL-10 production by DC stimulated with zymosan. Eur. J. Immunol. **37** (6), 1600–1612 (2007)
80. Goodridge, H.S., Simmons, R.M., Underhill, D.M.: Dectin-1 stimulation by candida albicans yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. J. Immunol. **178** (5), 3107–3115 (2007)
81. Underhill, D.M., Rossnagle, E., Lowell, C.A., Simmons, R.M.: Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. Blood **106**(7), 2543–2550 (2005)
82. Gringhuis, S.I., den Dunnen, J., Litjens, M., van der Vlist, M., Wevers, B., Bruijns, S.C., Geijtenbeek, T.B.: Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. Nat. Immunol. **10**(2), 203–213 (2009)
83. Goodridge, H.S., Shimada, T., Wolf, A.J., Hsu, Y.M., Becker, C.A., Lin, X., Underhill, D.M.: Differential use of CARD9 by dectin-1 in macrophages and dendritic cells. J. Immunol. **182**(2), 1146–1154 (2009)
84. Colonna, M., Samaridis, J., Angman, L.: Molecular characterization of two novel C-type lectin-like receptors, one of which is selectively expressed in human dendritic cells. Eur. J. Immunol. **30**(2), 697–704 (2000)
85. Sattler, S., Reiche, D., Sturtzel, C., Karas, I., Richter, S., Kalb, M.L., Gregor, W., Hofer, E.: The human C-type lectin-like receptor CLEC-1 is upregulated by TGF-beta and primarily localized in the endoplasmic membrane compartment. Scand. J. Immunol. **75**(3), 282–292 (2012)
86. Thebault, P., Lhermite, N., Tilly, G., Le Texier, L., Quillard, T., Heslan, M., Anegon, I., Soulillou, J.P., Brouard, S., Charreau, B., Cuturi, M.C., Chiffoleau, E.: The C-type lectin-like receptor CLEC-1, expressed by myeloid cells and endothelial cells, is up-regulated by immunoregulatory mediators and moderates T cell activation. J. Immunol. **183**(5), 3099–3108 (2009)
87. Turnbull, I.R., Colonna, M.: Activating and inhibitory functions of DAP12. Nat. Rev. **7**(2), 155–161 (2007)
88. Wang, L., Ren, S., Zhu, H., Hao, Y., Zhang, D., Ruan, Y., Zhou, L., Lee, C., Yun, X., Xie, J.: Structural and functional conservation of CLEC-2 with the species-specific regulation of transcript expression in evolution. Glycoconj J. (2012). In this issue
89. Xie, J., Wu, T., Guo, L., Ruan, Y., Zhou, L., Zhu, H., Yun, X., Hong, Y., Jiang, J., Wen, Y., Gu, J.: Molecular characterization of two novel isoforms and a soluble form of mouse CLEC-2. Biochem. Biophys. Res. Commun. **371**(2), 180–184 (2008)
90. Kerrigan, A.M., Dennehy, K.M., Mourao-Sa, D., Faro-Trindade, I., Willment, J.A., Taylor, P.R., Eble, J.A., Reis e Sousa, C., Brown, G.D.: CLEC-2 is a phagocytic activation receptor expressed on murine peripheral blood neutrophils. J. Immunol. **182**(7), 4150–4157 (2009)
91. Mourao-Sa, D., Robinson, M.J., Zelenay, S., Sancho, D., Chakravarty, P., Larsen, R., Plantinga, M., Van Rooijen, N., Soares, M.P., Lambrecht, B., Reis, E.S.C.: CLEC-2 signaling via Syk in myeloid cells can regulate inflammatory responses. Eur. J. Immunol. **41**(10), 3040–3053 (2011)
92. Chaipan, C., Soilleux, E.J., Simpson, P., Hofmann, H., Gramberg, T., Marzi, A., Geier, M., Stewart, E.A., Eisemann, J., Steinkasserer, A., Suzuki-Inoue, K., Fuller, G.L., Pearce, A.C., Watson, S.P., Hoxie, J.A., Baribaud, F., Pohlmann, S.: DC-SIGN and CLEC-2 mediate human immunodeficiency virus type 1 capture by platelets. J. Virol. **80**(18), 8951–8960 (2006)
93. Suzuki-Inoue, K., Fuller, G.L., Garcia, A., Eble, J.A., Pohlmann, S., Inoue, O., Gartner, T.K., Hughan, S.C., Pearce, A.C., Laing, G.D., Theakston, R.D., Schweighoffer, E., Zitzmann, N., Morita, T., Tybulewicz, V.L., Ozaki, Y., Watson, S.P.: A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2. Blood **107**(2), 542–549 (2006)
94. Watson, A.A., Brown, J., Harlos, K., Eble, J.A., Walter, T.S., O'Callaghan, C.A.: The crystal structure and mutational binding analysis of the extracellular domain of the platelet-activating receptor CLEC-2. J. Biol. Chem. **282**(5), 3165–3172 (2007)
95. Suzuki-Inoue, K., Kato, Y., Inoue, O., Kaneko, M.K., Mishima, K., Yatomi, Y., Yamazaki, Y., Narimatsu, H., Ozaki, Y.: Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. J. Biol. Chem. **282** (36), 25993–26001 (2007)
96. Kato, Y., Kaneko, M.K., Kunita, A., Ito, H., Kameyama, A., Ogasawara, S., Matsuura, N., Hasegawa, Y., Suzuki-Inoue, K., Inoue, O., Ozaki, Y., Narimatsu, H.: Molecular analysis of the pathophysiological binding of the platelet aggregation-inducing factor podoplanin to the C-type lectin-like receptor CLEC-2. Cancer Sci. **99**(1), 54–61 (2008)
97. Spalton, J.C., Mori, J., Pollitt, A.Y., Hughes, C.E., Eble, J.A., Watson, S.P.: The novel Syk inhibitor R406 reveals mechanistic differences in the initiation of GPVI and CLEC-2 signaling in platelets. J. Thromb. Haemost. **7**(7), 1192–1199 (2009)
98. Pollitt, A.Y., Grygielska, B., Leblond, B., Desire, L., Eble, J.A., Watson, S.P.: Phosphorylation of CLEC-2 is dependent on lipid

rafts, actin polymerisation, secondary mediators and Rac. Blood **115**(14), 2938–2946 (2010)
99. Fuller, G.L., Williams, J.A., Tomlinson, M.G., Eble, J.A., Hanna, S.L., Pohlmann, S., Suzuki-Inoue, K., Ozaki, Y., Watson, S.P., Pearce, A.C.: The C-type lectin receptors CLEC-2 and Dectin-1, but not DC-SIGN, signal via a novel YXXL-dependent signaling cascade. J. Biol. Chem. **282**(17), 12397–12409 (2007)
100. Dhanjal, T.S., Ross, E.A., Auger, J.M., McCarty, O.J., Hughes, C.E., Senis, Y.A., Buckley, C.D., Watson, S.P.: Minimal regulation of platelet activity by PECAM-1. Platelets **18**(1), 56–67 (2007)
101. Mori, J., Pearce, A.C., Spalton, J.C., Grygielska, B., Eble, J.A., Tomlinson, M.G., Senis, Y.A., Watson, S.P.: G6b-B inhibits constitutive and agonist-induced signaling by glycoprotein VI and CLEC-2. J. Biol. Chem. **283**(51), 35419–35427 (2008)
102. Wicki, A., Christofori, G.: The potential role of podoplanin in tumour invasion. Br. J. Cancer **96**(1), 1–5 (2007)
103. Suzuki-Inoue, K., Inoue, O., Ding, G., Nishimura, S., Hokamura, K., Eto, K., Kashiwagi, H., Tomiyama, Y., Yatomi, Y., Umemura, K., Shin, Y., Hirashima, M., Ozaki, Y.: Essential *in vivo* roles of the C-type lectin receptor CLEC-2: embryonic/neonatal lethality of CLEC-2-deficient mice by blood/lymphatic misconnections and impaired thrombus formation of CLEC-2-deficient platelets. J. Biol. Chem. **285**(32), 24494–24507 (2010)
104. Bertozzi, C.C., Schmaier, A.A., Mericko, P., Hess, P.R., Zou, Z., Chen, M., Chen, C.Y., Xu, B., Lu, M.M., Zhou, D., Sebzda, E., Santore, M.T., Merianos, D.J., Stadtfeld, M., Flake, A.W., Graf, T., Skoda, R., Maltzman, J.S., Koretzky, G.A., Kahn, M.L.: Platelets regulate lymphatic vascular development through CLEC-2-SLP-76 signaling. Blood **116**(4), 661–670 (2010)
105. Raica, M., Cimpean, A.M., Ribatti, D.: The role of podoplanin in tumor progression and metastasis. Anticancer. Res. **28**(5B), 2997–3006 (2008)
106. Chaipan, C., Steffen, I., Tsegaye, T.S., Bertram, S., Glowacka, I., Kato, Y., Schmokel, J., Munch, J., Simmons, G., Gerardy-Schahn, R., Pohlmann, S.: Incorporation of podoplanin into HIV released from HEK-293T cells, but not PBMC, is required for efficient binding to the attachment factor CLEC-2. Retrovirology **7**, 47 (2010)
107. Huysamen, C., Willment, J.A., Dennehy, K.M., Brown, G.D.: CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. J. Biol. Chem. **283**(24), 16693–16701 (2008)
108. Sancho, D., Mourao-Sa, D., Joffre, O.P., Schulz, O., Rogers, N.C., Pennington, D.J., Carlyle, J.R., Reis e Sousa, C.: Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. J. Clin. Invest. **118**(6), 2098–2110 (2008)
109. Caminschi, I., Proietto, A.I., Ahmet, F., Kitsoulis, S., Shin Teh, J., Lo, J.C., Rizzitelli, A., Wu, L., Vremec, D., van Dommelen, S.L., Campbell, I.K., Maraskovsky, E., Braley, H., Davey, G.M., Mottram, P., van de Velde, N., Jensen, K., Lew, A.M., Wright, M.D., Heath, W.R., Shortman, K., Lahoud, M.H.: The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. Blood **112**(8), 3264–3273 (2008)
110. Joffre, O.P., Sancho, D., Zelenay, S., Keller, A.M., Reis e Sousa, C.: Efficient and versatile manipulation of the peripheral CD4+ T-cell compartment by antigen targeting to DNGR-1/CLEC9A. Eur. J. Immunol. **40**(5), 1255–1265 (2010)
111. Lahoud, M.H., Ahmet, F., Kitsoulis, S., Wan, S.S., Vremec, D., Lee, C.N., Phipson, B., Shi, W., Smyth, G.K., Lew, A.M., Kato, Y., Mueller, S.N., Davey, G.M., Heath, W.R., Shortman, K., Caminschi, I.: Targeting antigen to mouse dendritic cells via Clec9A induces potent CD4 T cell responses biased toward a follicular helper phenotype. J. Immunol. **187**(2), 842–850 (2011)
112. Sancho, D., Joffre, O.P., Keller, A.M., Rogers, N.C., Martinez, D., Hernanz-Falcon, P., Rosewell, I., Reis e Sousa, C.: Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature **458**(7240), 899–903 (2009)
113. Schreibelt, G., Klinkenberg, L.J., Cruz, L.J., Tacken, P.J., Tel, J., Kreutz, M., Adema, G.J., Brown, G.D., Figdor, C.G., de Vries, I.J.: The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3+ myeloid dendritic cells. Blood **119**(10), 2284–2292 (2012)
114. Ahrens, S., Zelenay, S., Sancho, D., Hanc, P., Kjaer, S., Feest, C., Fletcher, G., Durkin, C., Postigo, A., Skehel, M., Batista, F., Thompson, B., Way, M., Reis, E.S.C., Schulz, O.: F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. Immunity **36**(4), 635–645 (2012)
115. Zhang, J.G., Czabotar, P.E., Policheni, A.N., Caminschi, I., San Wan, S., Kitsoulis, S., Tullett, K.M., Robin, A.Y., Brammananth, R., van Delft, M.F., Lu, J., O'Reilly, L.A., Josefsson, E.C., Kile, B.T., Chin, W.J., Mintern, J.D., Olshina, M.A., Wong, W., Baum, J., Wright, M.D., Huang, D.C., Mohandas, N., Coppel, R.L., Colman, P.M., Nicola, N.A., Shortman, K., Lahoud, M.H.: The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. Immunity **36**(4), 646–657 (2012)
116. Bakker, A.B., van den Oudenrijn, S., Bakker, A.Q., Feller, N., van Meijer, M., Bia, J.A., Jongeneelen, M.A., Visser, T.J., Bijl, N., Geuijen, C.A., Marissen, W.E., Radosevic, K., Throsby, M., Schuurhuis, G.J., Ossenkoppele, G.J., de Kruif, J., Goudsmit, J., Kruisbeek, A.M.: C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia. Cancer Res. **64**(22), 8443–8450 (2004)
117. Han, Y., Zhang, M., Li, N., Chen, T., Zhang, Y., Wan, T., Cao, X.: KLRL1, a novel killer cell lectinlike receptor, inhibits natural killer cell cytotoxicity. Blood **104**(9), 2858–2866 (2004)
118. Marshall, A.S., Willment, J.A., Lin, H.H., Williams, D.L., Gordon, S., Brown, G.D.: Identification and characterization of a novel human myeloid inhibitory C-type lectin-like receptor (MICL) that is predominantly expressed on granulocytes and monocytes. J. Biol. Chem. **279**(15), 14792–14802 (2004)
119. Marshall, A.S., Willment, J.A., Pyz, E., Dennehy, K.M., Reid, D.M., Dri, P., Gordon, S., Wong, S.Y., Brown, G.D.: Human MICL (CLEC12A) is differentially glycosylated and is down-regulated following cellular activation. Eur. J. Immunol. **36**(8), 2159–2169 (2006)
120. Pyz, E., Huysamen, C., Marshall, A.S., Gordon, S., Taylor, P.R., Brown, G.D.: Characterisation of murine MICL (CLEC12A) and evidence for an endogenous ligand. Eur. J. Immunol. **38**(4), 1157–1163 (2008)
121. Lahoud, M.H., Proietto, A.I., Ahmet, F., Kitsoulis, S., Eidsmo, L., Wu, L., Sathe, P., Pietersz, S., Chang, H.W., Walker, I.D., Maraskovsky, E., Braley, H., Lew, A.M., Wright, M.D., Heath, W.R., Shortman, K., Caminschi, I.: The C-type lectin Clec12A present on mouse and human dendritic cells can serve as a target for antigen delivery and enhancement of antibody responses. J. Immunol. **182**(12), 7587–7594 (2009)
122. Chen, C.H., Floyd, H., Olson, N.E., Magaletti, D., Li, C., Draves, K., Clark, E.A.: Dendritic-cell-associated C-type lectin 2 (DCAL-2) alters dendritic-cell maturation and cytokine production. Blood **107**(4), 1459–1467 (2006)
123. Kasahara, S., Clark, E.A.: Dendritic cell-associated lectin 2 (DCAL2) defines a distinct CD8alpha- dendritic cell subset. J. Leukoc. Biol. **91**(3), 437–448 (2012)
124. van Rhenen, A., van Dongen, G.A., Kelder, A., Rombouts, E.J., Feller, N., Moshaver, B., Stigter-van Walsum, M., Zweegman, S., Ossenkoppele, G.J., Jan Schuurhuis, G.: The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood **110**(7), 2659–2666 (2007)
125. Galligan, C.L., Baig, E., Bykerk, V., Keystone, E.C., Fish, E.N.: Distinctive gene expression signatures in rheumatoid arthritis

synovial tissue fibroblast cells: correlates with disease activity. Genes Immun. **8**(6), 480–491 (2007)
126. Michou, L., Cornelis, F., Levesque, J.M., Bombardieri, S., Balsa, A., Westhovens, R., Barrera, P., Alves, H., van de Putte, L., Migliorini, P., Bardin, T., Petit-Teixeira, E., Fernandes, M.J.: A genetic association study of the CLEC12A gene in rheumatoid arthritis. Joint Bone Spine (2012)
127. Zhang, H., Luo, J., Li, Y., Henderson, P.T., Wang, Y., Wachsmann-Hogiu, S., Zhao, W., Lam, K.S., Pan, C.X.: Characterization of high-affinity peptides and their feasibility for use in nanotherapeutics targeting leukemia stem cells. Nanomedicine (2011)
128. Hoffmann, S.C., Schellack, C., Textor, S., Konold, S., Schmitz, D., Cerwenka, A., Pflanz, S., Watzl, C.: Identification of CLEC12B, an inhibitory receptor on myeloid cells. J. Biol. Chem. **282**(31), 22370–22375 (2007)
129. Sattler, S., Ghadially, H., Reiche, D., Karas, I., Hofer, E.: Evolutionary development and expression pattern of the myeloid lectin-like receptor gene family encoded within the NK gene complex. Scand. J. Immunol. **72**(4), 309–318 (2010)
130. Willmentl, J., Davies, j, Brown, G.: Characterisation of a novel C-type lectin-like receptor, MaH. Int. Immunol. Meet. Abstr. **22** (Suppl 1 Pt 2), ii58 (2010). doi:10.1093/intimm/dxq114