C-type lectin receptors: pioneers in carbohydrate recognition on microbes

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1. Abstract

C-type lectin receptors (CLRs) comprise a group of advanced receptors, expressing the ability to recognize carbohydrates on host and microbial cells in a Ca^{2+} dependent manner. The family of CLRs is roughly divided in transmembrane (TM) and soluble CLRs. Today, around 80 CLRs have been identified. These CLRs are responsible for the recognition of over a thousand glycans present on host and microbial cells. Here, we review the various structural compositions of TM and soluble CLRs, resulting in specific CLR-carbohydrate interactions. The different structural compositions may contribute to the sophisticated function of CLRs. Clarifying the exact mechanism of carbohydrate recognition by CLRs on the basis of structural conformations will improve our knowledge on host-microbe interactions and may contribute to the development of improved therapeutic interventions.

Key words: *C-type lectin receptor, transmembrane CLR, soluble CLR, structural conformation, carbohydrate*

2. Introduction

Glycosylation is a universal process, resulting in the presence of carbohydrates on almost all cell walls of living cells, including microbe and host cells. Glycosylation occurs on protein and lipid backbones, or as an independent process (*e.g.* polysaccharide capsules surrounding certain bacteria)[1]. Due to the high amount of carbohydrates present on microbes, it is conceivable that the first interaction between host and microbe is formed by host recognition of a microbial carbohydrate structure. These carbohydrate structures form microbial signatures, also called "pathogen-associated molecular patterns" (PAMPs). The recognition of these PAMPs by host cell receptors is a conventional way to trigger innate and adaptive immune responses. The protein family of C-type lectins comprises an extensive group of pattern recognition receptor (PRR) proteins involved in carbohydrate recognition[2]. (Figure 1.) Strictly taken, the term 'C-type lectin' refers to Ca^{2+}-dependent carbohydrate recognition. However, the typical C-type lectin fold present on all C-type lectins is not always involved in Ca^{2+}-dependent carbohydrate recognition, and is therefore referred to as C-type lectin like domain (CTLD). Over one thousand metazoan proteins have been identified expressing one or more CTLDs, performing different functions like innate immunity, phagocytosis, endocytosis, complement activation, and cell adhesion[2, 3]. In contrast with the divergent evolution of CTLDs, the characteristic C-type lectin fold is an example of convergent evolution; it depicts a similar topology in all C-type lectins, but originates from different sequence motifs[4]. The subgroup of C-type lectin receptors (CLR) is known to act mainly as Ca^{2+}-dependent carbohydrate recognition proteins. CLRs are present in a soluble form (*e.g.* collectins), or in a transmembrane form (*e.g.* DC-SIGN, Dectin-1, MR, Langerin)[2, 5]. In this review, we focus on both soluble and transmembrane (TM) families of CLRs.

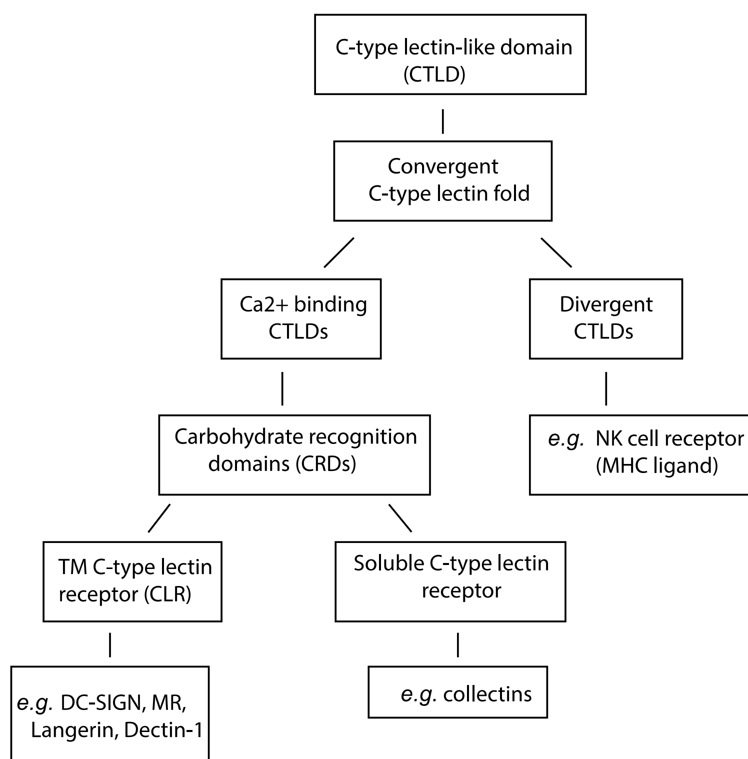

Figure 1. Schematic overview of the C-type lectin family nomenclature. TM; transmembrane, NK; natural killer, MHC; major histocompatibility complex.

Today the family of CLRs comprises around 80 receptor members. However, the exact number of CLRs is undefined since not all CLR ligands have been identified and thus these CLRs are only conceivable to function as Ca^{2+} dependent carbohydrate recognition receptors [1]. TM CLRs are present on a diverse group of myeloid cells, including various dendritic cell (DC) and macrophage subtypes, whereas the soluble CLRs are present as independent molecules that can act as opsonins to enhance phagocytosis[6]. The carbohydrate binding specificity of CLRs mainly resides within one or more carbohydrate recognition domains (CRD) present on the structural motif of the CTLD at the extracellular side of the protein. Ligand targeting of TM CLRs is often followed by regulated cell-cell communication, antigen uptake, and skewing of an appropriate immune response. Soluble CLRs are involved in the enhancement of distinct processes of innate immunity, including phagocytosis, cytokine secretion, and complement activation[7]. In contrast, CLRs also contribute to maintain self-tolerance by binding carbohydrates present on their own membranes[8].

The interaction between carbohydrates and CLRs usually forms a low affinity binding. However, low affinity is overcome by binding in a multivalent manner[9]. The enormous heterogeneity of oligosaccharides present in soluble form or on proteins and lipids suggests that carbohydrate recognition by CLRs is an advanced process[10]. In addition, for several CLRs only a few or no carbohydrate ligands are known today[11].

The biological importance of carbohydrates has been underestimated by the scientific community for years. This was partly due to the lack of standardized protocols for oligosaccharide synthesis and characterization[12]. Nowadays, more advanced techniques are available and the amount of research on carbohydrate-host interactions is increasing, improving carbohydrate knowledge to an equal basis with its counterparts such as proteins and nucleic acids. Here, we review the current knowledge on the diverse ways of CLR binding to their cognate carbohydrate ligands. The structural composition of both, soluble and TM CLRs is described. In addition, the most important CLRs are introduced and compared in order to elucidate how CLRs form various conformations resulting in different specific carbohydrate interactions. Clarifying the advanced and diverse process of carbohydrate recognition by CLRs on the basis of structural conformations might form the basis for promising future approaches concerning research on host-microbe interactions.

3. General composition of CLRs

The prototypic C-type lectin fold present in all CLRs is comprised of two anti-parallel β-strands and two α-helixes. However, the variety amongst CLRs lies in the difference of CTLDs and CRDs present on these CTLDs. CLRs express one or several CRDs on their CTLDs[13]. These CRDs recognize carbohydrates in a calcium-dependent manner. They are comprised of roughly 120 amino acids, among which 14 invariant amino acids and 18 conserved amino acids, confirming overlap in tertiary structure as well as distinct carbohydrate recognition properties[14]. The Ca^{2+} ion is involved in glycan binding and in maintaining the structural integrity of the CRD that is needed for appropriate function[4]. Five conserved amino acid residues present on the CRD have been demonstrated to be involved in Ca^{2+}-carbohydrate interaction. The presence of these five residues predicts interaction of two equatorial hydroxyl groups belonging to monosaccharides by two pairs of glutamic acid and asparagine residues at the principal Ca^{2+} site, resulting in classical Ca^{2+}-sugar binding[14]. CLRs can be categorized on the basis of conserved amino acid motifs that determine the preference of glycan recognition and Ca^{2+} ion coordination. CLRs that possess an EPN (Glu-Pro-Asn) amino acid motif in the CRD prefer binding and uptake of glycans with equatorial 3- and 4-hydroxyl (OH) groups like mannose, fucose, and/or *N*-acetylglucosamine (GlcNac) residues (*e.g.* DC-SIGN, MR), whereas CLRs that express a QPD (Gln-Pro-Asp) motif possess specificity for vertical 4-OH groups like galactose and *N*-acetylgalactosamine (GalNAc) terminated glycans (*e.g.* MGL)[15, 16]. Some exceptions are known, for example Dectin-1 expressing a putative β-glucan binding side with a hydrophobic character instead of an EPN or QPD motif. Therefore, Dectin-1 is dedicated a C-type lectin like receptor[17]. The majority of CLRs possesses the EPN amino acid motif, with beneficial result since EPN related glycans mannose, fucose, and GlcNAc residues are normally not found on mammalian cells, but abundant on surface proteins of microbes, for example on HIV-1 envelope protein gp120[18]. Despite the general structural composition of CLRs, these receptors also express various characteristic properties regarding their structural composition, resulting in a variety of specific CLR-carbohydrate interactions.

4. Transmembrane C-type lectin receptors

4.1 General composition of TM CLRs

TM CLRs are generally divided in type I and type II receptors on the basis of their molecular structure. The receptors of both groups express one or more extracellular CRDs, a stalk region, a TM region and an intracellular part with or without a signaling motif. Type I CLRs have their N terminus pointing outwards the cell, whereas type II CLRs have their N terminus pointing inwards the cytoplasm[10]. In addition, type I CLRs express various CRDs on their extracellular C-type lectin domains (CTLD), while on the contrary type II CLRs only possess single CRDs positioned at the C-terminus[10]. A schematic representation of the conformation of type I and type II CLRs is depicted in figure 2.

After ligand binding, signaling motifs present at or bound to the intracellular part of the CLRs translate the CLR-carbohydrate interaction into a specific signaling cascade. Within the family of CLRs different signaling motifs have been elucidated. Spleen tyrosine kinase (Syk) is the proximal adaptor of most CLRs. Syk is able to bind proteins expressing immunoreceptor tyrosine-based activation motifs (ITAMs). Phosporylation of two tyrosines within the ITAM motif by Src kinases forms a docking site for the tandem SH2 domains of Syk. Syk binding to the ITAM is followed by a conformational change causing phosphorylation and activation of various substrates resulting in a signaling cascade[19]. CLRs often couple to Syk via association with ITAM-expressing adaptors. For example, Dectin-2, BDCA-2 and Mincle are able to associate with the Fc receptor γ (FcRγ) chain, an adaptor protein expressing an ITAM[20]. In contrast, some CLRs bind Syk directly via a single tyrosine-based motif present at the intracellular domain, this single tyrosine-based motif is termed ITAM-like. Dectin-1 was the first CLR demonstrated to express an ITAM-like motif [17]. Another group of CLR signaling domains is characterized by the presence of an immunoreceptor tyrosine-based inhibitory motif (ITIM) at the intracellular domain. For instance, the Dectin-2 family receptor DCIR expresses an ITIM. These ITIMs are comparable to hemITAMs, except ITIMs recruit phosphatases (*e.g.* SHP-1, SHP-2, and SHIP) instead of kinases[19]. Signaling upon CLR-carbohydrate binding results in for instance gene transcription, regulated antigen processing, and endocytosis. However, limited evidence is present whether independent CLR signaling is sufficient to induce microbial effector function. It is conceivable that CLRs co-operate with other antigen sensing proteins in order to fine-tune the immune response. For instance Dectin-I has been proven to co-operate with TLR2 and TLR4. Synergistic increase of TNF-α was measured in monocyte-derived macrophages when the three receptors were simultaneously stimulated[21]. In addition, Dectin-1 enables Syk-dependent signaling of the murine CLR SIGNR-1 resulting in an oxidative response to *C. albicans*[22]. CLR MGL does not express an ITAM-like motif, and neither binds to an ITAM expressing adaptor. However, MGL has been demonstrated to interact with certain toll-like receptors (TLRs) in order to enhance IL-10 and TNF-α production[23].

As discussed in this section, the diverse groups of type I and II TM CLRs possess structural overlap as well as structural differences. These variations result in diverse binding of CLRs to carbohydrates and subsequent (immune) responses. Additional characteristics of the structural composition and some prominent examples of type I and II TM CLRs are described in the next sections.

4.2 Type I CLRs

The mannose receptor (MR) family comprises the most prominent group of type I CLRs. Within the family of MRs, the mannose receptor (MR) and Endo180 receptor have been demonstrated to express carbohydrate-binding activity[24]. (Table 1.) Both receptors are present on phagocytic cells and possess a unique composition of eight CRDs present on a single polypeptide backbone, a N-terminal cysteine-rich domain, a single fibronectin type II (FNII) domain, and a short cytoplasmic domain[24, 25]. (Figure 2.) A conceivable C-terminal signaling motif enhances endocytic properties promoting the uptake of ligands into the cell upon receptor-ligand interaction. After endocytosis, recycling of the receptor to the cellular membrane may occur. The endocytosis and recycling process does not occur via an ITAM-like motif. It is conceivable that a specific recycling motif is involved. However, the exact signaling remains elusive[24, 26]. Closely packed fibronectin type II repeats are involved in antigen transport and enhancement of cell-cell interactions[10]. Both receptors are able to bind mannose, fucose, and *N*-acetylglucosamine (GlcNac) in a Ca^{2+} dependent way, MR via CRD4 and CRD8 and Endo180 via CRD2[18, 27].

Only carbohydrate binding via CRD4 and CRD8 of the MR has been demonstrated with isolated recombinant MR. However, the five residues known to cross-link the principal Ca^{2+} are also present in a conserved manner in CRD5 of the MR. It is likely that, besides CRD4 and CRD8, CRD5 is involved in carbohydrate binding *in vivo* as well. Within the Endo180 CLR, only CRD2 contains all five residues required for binding the carbohydrate and Ca^{2+}, confirming that only CRD2 has sugar-binding properties[27].

Two monomeric conformations of the MR have been identified, a "bent" form, and a more extended MR form, indicating flexible MR conformation[28]. Within the "bent" form, interactions between the eight CRDs and the Cys-RDs of MR form a network favorable for mannose residue binding. The linker regions of CRD3 and CRD6 (surrounding CRD4 and CRD5) are flexible exposed. This results in a close separated contact between CRD4 and CRD5, forming a ligand-binding core in the center of the polypeptide. This "bent" rearrangement enhances binding of multiple mannose residues of high mannose oligosaccharides[27]. The Endo180 receptor expresses a similar conformation as MR. However, only CRD2 contains Ca^{2+}-ligand cross-linking properties, resulting in a different binding conformation[27]. Apparently, the open and "bent" conformations of Endo180 and MR fluctuate depending on pH levels. Upon CLR-ligand binding on the cell-membrane, endocytosis of the complex may occur and translocation of the CLR-ligand complex to an endosomal compartment takes place prior to antigen persentation. Decreased pH within the endosomal compartment alters Ca^{2+} affinity resulting in loss of Ca^{2+} from the CLR, with subsequent loss of CLR ligand binding. Loss of Ca^{2+} is accompanied by alteration of receptor conformation from a compact to a more open conformation[28, 29]. Thus, flexible conformations of MR and Endo180 contribute to efficient CLR-ligand binding and subsequent immune regulation.

In addition, an equilibrium between monomeric and dimeric mannose receptor (MR) conformations is present at the surface of primary monocyte-derived DCs and macrophages[30]. Both monomer and dimer MR complexes are able to bind mannose residues. Strikingly, MR dimerization appeared to be essential for binding envelope glycoproteins gp120, gp140, and HIV-1 viral particles. gp120 binding to MR occurred in a Ca^{2+}dependent and Ca^{2+}independent manner, namely via its cysteine-rich domain (Cys-RD)[18]. Apparently, upon the formation of dimeric complexes, the clustering of CLRs is positioned in an altered and more appropriate distance for enhanced

carbohydrate recognition.

A remarkable difference between MR and Endo180 is the property of MR to bind sulfated sugars via its cysteine-rich domain in contrast to Endo180 [26]. Thus, although the MR and Endo180 are both designated type I TM mannose receptors, it is conceivable that *in vivo* both receptors have distinct sets of glycoprotein ligands due to differences in structural conformation and receptor multimerization. In addition to MR and Endo180 more type I CLRs within the MR family are known, e.g. DEC-205 and the M-type phospholipase A_2 receptor. However, these receptors are not involved in carbohydrate recognition or their ligand specificity is still undefined[10, 24].

4.3 Type II CLRs

The majority of TM CLRs is designated to the group of type II CLRs. (Table 1.) As depicted in figure 2, type II CLRs are composed of a short N-terminal cytoplasmic tail, a TM domain, an extracellular stalk region, and a CTLD expressing a single CRD. The length of the extracellular stalk region varies among the different type II CLRs[31]. The majority of CLRs present on DCs are type II TM proteins. Upon maturation, DCs downregulate expression of their CLRs[1].

DC-specific ICAM3-grabbing non-integrin (DC-SIGN) is the most extensively studied type II CLR. DC-SIGN contains highest affinity for mannose and fucose-containing glycans found on certain microbial surfaces and on Lewis blood group antigens in human tissue[32, 33]. DC-SIGN possesses preference for binding oligosaccharides linked in an α-anomeric way. For example, the HIV-1 envelope glycoprotein gp120 contains a high amount of mannose type glycans that are linked in an α-anomeric way, explaining the high affinity binding between gp120 and DC-SIGN[34]. The diverse set of microbes recognized by DC-SIGN includes HIV-I, *Candida albicans*, *Mycobacterium tuberculosis*, ebola virus, and hepatitis C virus[35]. In addition to microbial ligands, DC-SIGN has been demonstrated to bind the human endogenous ligands ICAM-2 and ICAM-3 [34]. Receptor oligomerization of DC-SIGN occurs through extended neck region and is facilitated via the formation of α-helical coiled coils. The extended neck region composed of eight 23 amino acid repeats is encoded on a single exon on which polymorphism has been demonstrated. Alleles with 4 to 9 repeats in the DC-SIGN neck region-coding exon have been elucidated as well [36]. Heterogeneity at the neck region-coding exon correlates with alterations in DC-SIGN structural conformation and influences the affinity and specificity for carbohydrate recognition[37]. In addition, the CRD of DC-SIGN is linked to the neck region in a flexible way, allowing the receptor to interact with glycans that express different virulent surfaces and spacings[33, 35]. The presence of at least two 23 amino acid repeats within the neck region is sufficient for DC-SIGN to form tetramers on the cell surface, resulting in improved carbohydrate binding [33, 36]. Another variable contributing to the dynamic structural composition of DC-SIGN is the coordination of the principal Ca^{2+} molecule within the CLR. This coordination is determined by hydrogen bonds formed by hydroxyl groups of pyranose rings with the amino acid side chains. Each coordination of the Ca^{2+} molecule features typical CLR binding to a cognate glycan ligand[38]. Moreover, DC-SIGN expresses two Ca^{2+} binding positions within its CRD: Ca^{2+} binding site 1 and 2. DC-SIGN ligand binding to Ca^{2+} site 2 occurs via several conformations. The Ca^{2+} site 1 forms the so-called auxiliary site. This Ca^{2+} site 1 enhances the formation of a correct positioning of the loops forming the primary ligand-binding site (Ca^{2+} site 2)[39]. Although DC-SIGN expresses an ITAM-like motif on the intracellular part, only Ras signaling upon DC-SIGN activation has been demonstrated. This Ras signaling occurs independent of the ITAM-like motif [19]. Glycan ligand moiety and type are

also influencing the regulation of the DC-SIGN signalosome. Distinct DC-SIGN signaling pathways are induced upon binding by mannose or fucose-expressing microbial agents to the same Ca^{2+} binding site. *Mycobacterium tuberculosis* and HIV-1 virus type both express mannose, by binding to DC-SIGN the mannose moiety enhance recruitment of effector proteins to the DC-SIGN signalosome in order to activate the kinase Raf-1. In contrast, fucose-expressing *Helicobacter pylori* promoted dissociation of the KSR1-CNK-Raf-1 complex from the signalosome of DC-SIGN[40]. Thus, mannose- and fucose-expressing expressing microbial agents have a different effect on the regulation of the signalosome, resulting in activation or suppression of the pro-inflammatory response respectively.

The Langerhans cell (LC) related CLR langerin expresses a similar domain organization as DC-SIGN and is also known to recognize mannose and fucose-containing glycan residues. Both the CRDs of DC-SIGN and langerin express the five conserved amino acid residues involved in Ca^{2+}-sugar binding. In contrast to DC-SIGN, trimerization instead of tetramerization of the neck region of langerin occurs to enhance carbohydrate binding[29]. The intracellular part of langerin contains a proline-rich signaling motif. This proline rich motif functions as a docking site for signaling proteins, for instance the SH2 domains present on Syk. The presence of an ITAM-like motif at the langerin receptor has never been demonstrated [41].

Unlike the mannose- and fucose- recognizing CLRs, only a single galactose recognizing CLR is known within the immune system: the so-called macrophage galactose-type lectin (MGL). Human MGL is a type II CLR with a conserved QPD (Gln-Pro-Asp) motif. This CLR is expressed exclusively as a homo-oligomer on myeloid DCs and macrophages[42, 43]. MGL solely expresses specificity for terminal GalNAc residues. These rare GalNAc terminal moieties are found on glycans expressed by *C. jejuni*, tumor-associated mucin MUC1, and on effector T cells CD45, implicating the involvement of MGL in the control of adaptive (tumor) immunity[44, 45]. No ITAM-like motif has been found on the intracellular part of MGL. However, MGL has been demonstrated to enhance IL-10 and TNF-α levels upon binding and co-functioning with various TLRs[23].

4.4 TM C-type lectin like receptors

Despite the well characterized type I and type II TM CLRs mentioned previously, an extensive group of TM CLRs belonging to the families of Dectin-1 and Dectin-2 receptors are still poorly characterized. The genes of the Dectin-1 and Dectin-2 CTLRs are all grouped on the NK gene cluster of human chromosome 12 [46, 47]. Strictly taken, Dectin-1 and Dectin-2 receptor members belong to the group of C-type lectin like receptors since Ca^{2+} dependent carbohydrate recognition has not been proven for these receptors[10, 48]. All receptors are expressed on various myeloid cells in human individuals and express the classical type II CLR conformation consisting of a single extracellular CTLD, a stalk region, a TM domain and an intracellular region with or without expressing of a signaling motif [46, 47].

The dendritic cell-associated C-type lectin-1 (Dectin-1) family of CTLRs comprises C-type lectin like domain containing-1 (CLEC-1), CLEC-2, CLEC-9A, Dectin-1, myeloid inhibitory C-type-like lectin (MICL), macrophage antigen H (MAH) and Lectin-like Oxidised LDL receptor-1 (LOX-1)[47]. Dectin-1, the most well studied CLR of the Dectin-1 family, is known for its role in mediating innate immunity against fungi. Dectin-1 is able to bind β-glucan-rich extracts present at microbial agents such as *Saccharomyces cervisiae*. Dectin-I contains a short neck region, resulting in a monomeric state on the cell surface[49]. CLEC-2, CLEC-9A and LOX-1 are binding to alternative

ligands rather than the classical carbohydrate ligands. CLEC-2 binds to sialoglycoprotein and rhodocytin, CLEC-9A to F-actin present on apoptotic cells and LOX-1 to lipid structures. Ligands of CLEC-I, MICL and MAH are yet to be identified[47].

The Dectin-2 family of CTLRs includes dendritic cell immunoreceptor (DCIR), Dectin-2, C-type lectin superfamily 8 (CLECSF8) also named Dectin-3, macrophage-inducible C-type lectin (Mincle), and blood dendritic cel antigen 2 (BDCA-2)[46]. Mincle, BDCA-2 and Dectin-2 do all express an EPN motif resulting in sensitivity for mannose-ligands. In addition, Mincle and Dectin-2 both interact with microbial bacteria, for instance interaction with *C. albicans* has been demonstrated for both receptors. DCIR also expresses an EPN motif. However, carbohydrate binding to the DCIR receptor is never reported[46]. CLECSF8/Dectin-3 has been recently demonstrated to bind α-mannan structures[20].

Further elucidation of the composition and functioning of the CLRs within the Dectin-1 and Dectin-2 clusters is useful in order to further enhance the current knowledge on CLR functioning. Current research on the Dectin-1 and Dectin-2 families encompasses not only the investigation of the independent CLR compositions and functions, but focuses in an increased manner on the co-working of these CLRs resulting in increased sensitivities towards microbial agents. For example, CLRs Dectin-2 and Dectin-3 both recognize α-mannan structures on the surface of *C. albicans* with subsequent induction of NF-κB-mediated Th17 and Th1 responses through Syk signaling. Apperently, Dectin-2 and Dectin-3 constitutively form heterodimers on the cell surface of myeloid cells. Improved sensitivity of the host cell to sense *C. albicans* infection was measured by the Dectin-2 and Dectin-3 heterodimers compared to the respective homodimers[20]. Other recent studies confirmed a contrary role for the Dectin-2 family CLR mincle. Mincle and Macrophage C-type lectin (MCL) have been demonstrated to form heterodimers on the cell surface in a rat cell line. Association of MCL with the ITAM-expressing adaptor FcεRI-γ increased Mincle expression. Subsequently, the mincle/MCL/FcεRI-γ complex enhances phagocytosis of Ab-coated beads, compared to complexes lacking one of the components[50]. Previous observation confirms a pro-inflammatory role for mincle when binding to the MCL protein. In contrast, mincle appeared to be able to express an anti-microbial response as well upon binding to *F. monophora*. Moreover, Interferon regulatory factor 1 (IRF1) induces nucleosome remodeling of *IL12A* and is crucial for IL-12 production. Dectin-1 binding to *F. monophora* induces IRF1-dependent IL-12A mRNA expression. Simultaneously, CLR mincle is able to bind *F. monophora* resulting in specific degradation of IRF1 via E3 ubiquitin ligase Mdm2. IRF1 degradation results in suppression of *IL12A* transcription. Thus, mincle is involved in antifungal immunity by suppressing IL-12 production[51]. Previous examples of CLRs that co-operate to induce enhanced sensitivity suggest that there might be other CLRs working together to improve their sensing mechanisms. However, the lack of knowledge on structural composition and ligand binding of several CLRs is working against the attempt to find more co-operating CLRs.

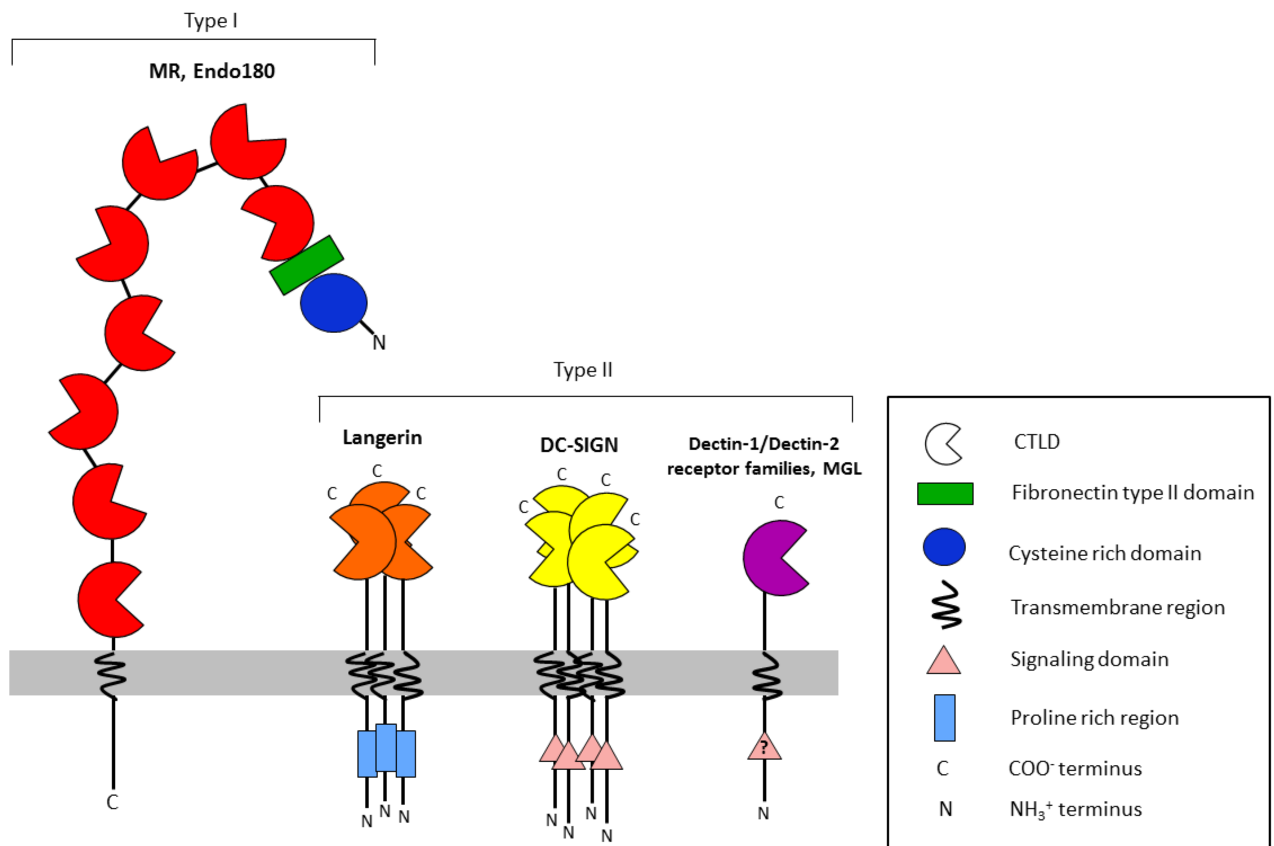

Figure 2. Schematic representation of type I and type II C-type lectins and lectin-like receptors. Type I CLRs (MR, Endo180) are composed of an N-terminal cysteine-rich domain, a single fibronectin type II (FNII) domain, eight CTLDs all expressing CRDs, a TM region, and a cytoplasmic domain with a signaling motif. Type II CLR (Langerin, DC-SIGN, MGL) or lectin-like receptors (Dectin-1 and Dectin-2 receptor families) are composed of a single CTLD, an extracellular stalk region, a TM region, and a N-terminal cytoplasmic tail with or without a signaling motif or proline rich region. Langerin, DC-SIGN, MGL, and Dectin-1 express a CRD on their CTLD. CTLD, C-type lectin like domain; CLR, C-type lectin receptor; CRD, carbohydrate recognition domain; MR, mannose receptor; DC-SIGN, DC-specific ICAM3-grabbing non-integrin; MGL, macrophage galactose-type lectin.

Table 1. Characteristics of TM C-type lectin and lectin-like receptors.

TM C-type lectin receptor	Type I or II	Present on*	Ligands	Conformation on cell surface	Signaling motif
MR	I	DCs, LCs, Mo, Mϕ	Mannose, fucose, GlcNac	Monomers/dimer	ND
Endo 180	I	DCs, LCs, Mo, Mϕ	Mannose, fucose, GlcNac	Monomers	ND
DC-SIGN	II	DCs, Mo, Mϕ	Mannose, fucose, GlcNac	Tetramers	ITAM-like
Langerin	II	LCs	Mannose, fucose	Trimers	Proline-rich region
MGL	II	Mϕ, DCs, ECs	Galactose, GalNac	Monomers	ND
Dectin-I	II	DCs and LCs	β-glucan	Monomers	ITAM-like
CLEC-I	II	DCs, LCs, ECs	ND	Dimers	ND
CLEC-2	II	DCs, Mo, Gra, NKs, platelets	Sialoglycoprotein, rhodocytin	Tetramers	ITAM-like
CLEC-9A	II	DCs	F-actin	Monomers/dimers	ITAM-like
MICL	II	DCs, Mϕ	ND	Monomers	ITIM
MAH	II	DCs, Mo, Lym	ND	Monomers	ITIM
LOX-I	II	DCs, Mϕ, ECs, Mo, B cells	Lipid structures	Dimers	Asp-Asp-Leu (DDL) motif
Dectin-2	II	DCs, LCs, Mo	Mannose, fucose, GlcNac	Monomers/dimers	FcRγ adaptor
DCIR	II	Dcs, Mo, Gra, B cells	Fucose, mannose?	ND	ITIM
CLECSF8/Dectin-3	II	DCs and Mo	α-mannose	Dimers	ND
Mincle	II	DCs, Mo, Mϕ, B cells	α-mannose, fucose, GlcNac	ND	FcRγ adaptor
BDCA-2	II	DCs	Mannose, fucose, GlcNac, asialo-galactosyl	ND	FcRγ adaptor

* DC, dendritic cell; LC, Langerhans cell; Mo, monocyte; Mϕ, macrophage; EC, endothelial cell; Gra, granulocytes; Lym, lymphocytes; NK cells, natural killer cells; ND, not defined.

5. Soluble C-type lectin receptors

5.1 Soluble CLRs: the family of collectins

Soluble CLRs form a subclass of lectins with a characteristic composition and function due to their soluble presence within the plasma and mucosal surfaces. Recognition of microbes by soluble CLRs may result in removal of the microbes via opsonization, complement activation, initiation of phagocytosis or inhibition of microbial growth. Soluble CLRs are also involved in modulating adaptive immune responses by forming connections between antigen presenting cells and the carbohydrates present on the microbes[7]. Soluble CLRs lack signaling motifs on their structural compositions. Instead, various immune responses can be triggered after opsonization by phagocytic cells[7].

All well-known soluble C-type lectins belong to the family of collectins (collagen-containing C-type lectins), including human collectins mannose-binding lectin (MBL), lung surfactant proteins A (SP-A), SP-D, CL-L1, CL-P1, and CL-K1[7]. Collectins are macromolecules, spanning seizes of up to 100 nm. This wide conformation provides advantages for the agglutination of microbial agents. Functionally, collectins are assembled as trimers of three identical polypeptide chains, as depicted in figure 3A and B. The formation of trimeric subunits is required for high affinity binding. The single polypeptide chains of collectins have a similar modular domain composition composed of four parts; a short cysteine-rich N-terminal domain followed by a collagen-like region, an α-helical neck domain, and a C-terminal CRD[7]. The collagen-like region is composed of repeating Gly-Xaa-Yaa amino acid triplets that form triple helices with various lengths. The neck domain region formed of α-helical coiled-coils is essential for the formation of the collagen-like triple helices, arranging the collagen chains that eventually result in a "zipper-like" folding of the triple helix[52]. The triple helices provide strength and resistance to self and non-self proteases. Multimerization of the triple helices occurs in various orders among the different collectins via the formation of disulphide bonds by the cysteines in the N-terminal region. In general, two common types of multimerization can be distinguished: the cruciform type and the bouquet type. (Figure 3C) For example, collectins SP-A and MBP are composed of six trimeric subunits that form octadecamers. Interruptions in the Gly-Xaa-Yaa amino acid triplets 13 of SP-A and 8 of MBP by a so-called break sequences result in bend structures due to the angling of the domains away from the central core. These tertiary structures are reminiscent of a bouquet of flowers. The bend bouquet-like structure provides increased flexibility[53]. In contrast, the post-translational conformation of SP-D is formed by four trimeric subunits that form a dodecamer reminding of a cruciform-like structure. SP-D is also able to form higher multimers[54]. However, the exact structures of these variable higher order multimers are yet to be identified[7]. The formation of multimeric complexes is an efficient way of combining forces to enhance density-dependent carbohydrate recognition. When comparing the structural composition of TM CLRs to soluble CLR compositions, we can suggest that TM CLRs form a high density receptor pattern on the host cell membrane enhancing a strong interaction on a small surface. In contrast, soluble CLRs tend to form more widely stretched multimeric conformations in order to surround microbial agents.

5.2 SP-A and SP-D

Lung surfactant proteins –A and –D are found in surfactant present at the luminal surface of pulmonary epithelial cells. Besides binding mannose residues, SP-A proteins can bind fucose with higher affinity[55]. Human SP-A trimers are composed of two sub polypeptide chains: SP-A1 and SP-A2. Two SP-A1 molecules together with one SP-A2 molecule form heterotrimeric subunits. SP-A1 and SP-A2 subtypes have a slightly different composition resulting in different function, at amino acid number 223 SP-A1 expresses a glutamine and SP-A2 expresses a lysine. Glutamine is neutral whereas lysine is a positively charged amino acid. This altered amino acid contributes to enhanced affinity binding of SP-A2 to mannose and fucose compared to SP-A1[55, 56]. SP-A has been demonstrated to be involved in the regulation of surfactant phospholipid pools in alveolar cavities via CRD-dependent phospholipid binding[53]. In addition, SP-A can interact with lipids via its collagen-like region. The role of SP-A within immune regulation remains controversial. First, SP-A forms bouquet-like octadecamers via the formation of intermolecular disulphide bonds at the N-terminus. Afterwards, further self-association may occur via reversible Ca^{2+}-dependent supraquaternary structure formation. These complex supraquaternary structures bind to phospholipids and other protein structures and enhance immunological functions and biophysical effects, for example the reduction of surface tension at the air-liquid interface by binding dipalmitoylphophatidylcholine (DPPC)[57].

The SP-D lectin receptor binds to maltose, mannose, and glucose carbohydrates. In addition, rough lipopolysaccharides of Gram-negative bacteria are recognized via the SP-D CRDs. For example, Non-capsulated *Klebsiella pneumonia* expressing mannose-containing O-antigens was proved to be rapidly opsonized by SP-D, followed by IL-1β and IL-6 production by macrophages and eradication of the bacterium in mice [58]. In addition, The CRD of SP-D is able to bind high-mannose oligosaccharides present on the hemagglutinin (HA) coat on the majority of the influenza strains[59]. Besides carbohydrate recognition, SP-D has been demonstrated to bind phosphatidylinositol and glucosylceramide[60].

5.3 MBL

Mannose binding lectin (MBL) is a serum collectin produced by the liver, expressing EPN motif with binding-preference for mannose, fucose, and GlcNAc carbohydrate residues. Trimeric MBL structures are circulating in the plasma and most likely form 'bouquet-like' octadecamers. In addition, other lower or higher order MBL conformations have also been detected within serum (*e.g.* trimers and tetramers)[61]. The three CRDs that are present within the triple helix are separated from each other at a constant distance (45 Å in human), providing a conformation to recognize multiple glycans at the same time[62]. Three well-defined MBL polymorphisms are known, on codon 52, 54, and 57 [62]. These polymorphisms cause amino acid replacement of arginine for cysteine, glycine for aspartic acid and glycine for glutamic acid respectively in the collagen domain. These differences in amino acid sequence alter the ability of MBL to oligomerize. In addition, two common polymorphisms are identified in the upstream promoter region of MBL. These polymorphisms result in a wide range of MBL serum levels among different individuals. Low MBL serum levels have been associated with various diseases including immune compromised diseases [63]. MBL is the only collectin demonstrated to be able to initiate activation of the lectin pathway of the complement system. MBL associates to MBL-associated serine proteases (MASP-1, MASP-2, and MASP-3), *e.g.* MBP binding to MASP-2 yields a complex that triggers complement C2 and C4 cleavage[64].

5.4 CL-L1, CL-KI, and CL-PI

Besides the so-called 'classical collectins' SP-A, SP-D, and MBL that are predominately involved in first-line host defense, three 'novel collectins' were identified within human; collectin liver 1 (CL-L1), collectin kidney 1 (CL-K1), and collectin placenta 1 (CL-P1)[65-67]. CL-L1 is a collectin expressed in the cytoplasm, binding to D-mannose, L-fucose, and D-galactose[66]. CL-P1 is expressed on vascular endothelial cells and acts as a scavenger receptor, by carbohydrate-dependent binding to microorganisms such as *E. Coli*, *Staphylococcus aureus*, and yeast [65]. CL-K1 is very similar to CL-L1 in terms of amino acid sequence despite that CL-K1 is secreted while CL-1 resides within the cytoplasm. CL-KI has been demonstrated to bind L-fucose, and D-mannose[67]. Exact functioning of these 'novel collectins' still needs to be identified.

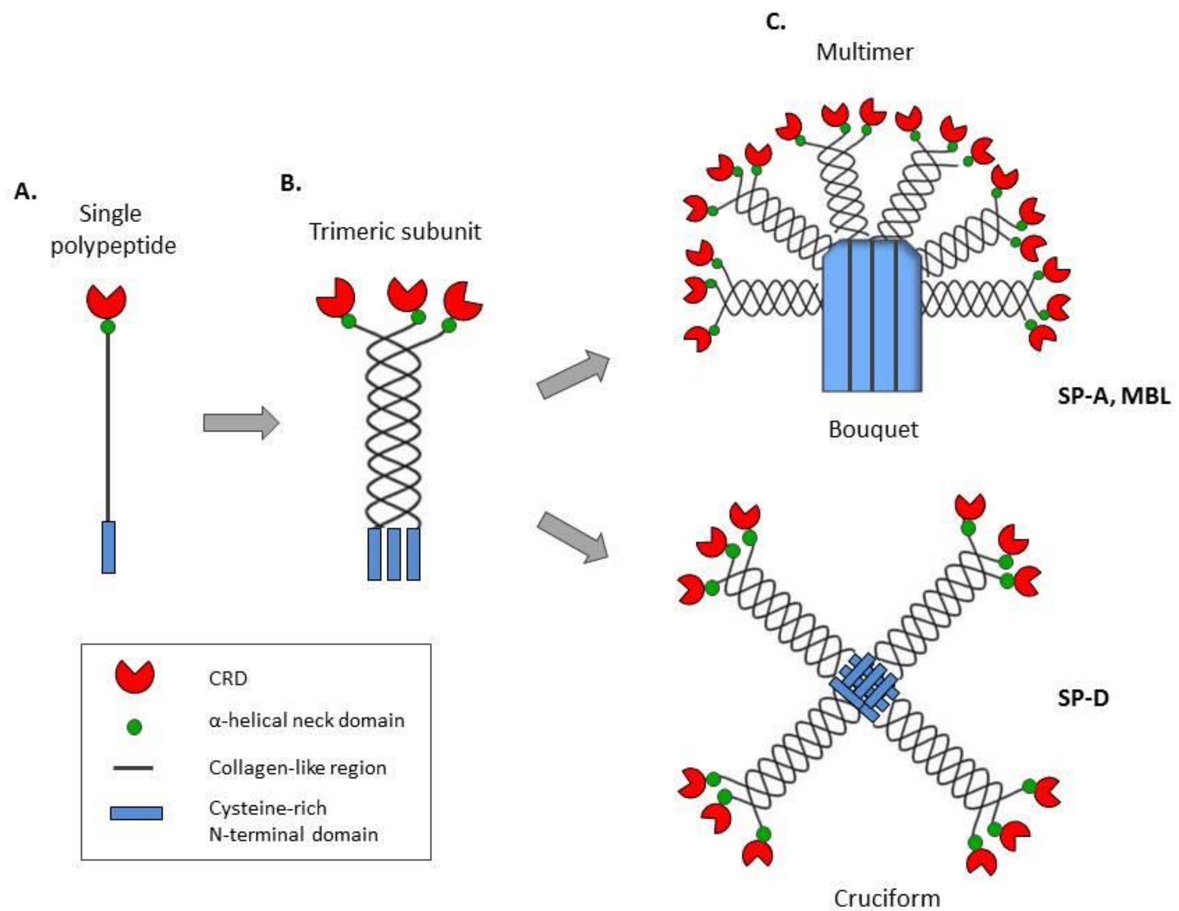

Figure 3. Schematic representation of different soluble CLR conformations. (C.) Two types of soluble CLR macromolecule conformations can be distinguished; the 'bouquet' conformation (SP-A, MBP), and the 'cruciform' type (SP-D) formed via hydrophobic interactions and N-terminal disulphide-bonds. **(B.)** These conformations respectively exist of six and four trimeric subunits. The trimeric subunits are composed of three polypeptide chains. **(A.)** The single polypeptide chains are the primary structures consisting of a C-terminal CRD, an α-helical neck domain, a collagen-like region, and a cysteine-rich N-terminal domain. SP-A, surfactant protein-A; SP-D, surfactant protein-D; MBP, mannose binding lectin; CRD, carbohydrate recognition domain.

6. Concluding remarks

CLRs comprise a group of advanced receptors possessing the ability to recognize carbohydrates in a Ca^{2+} dependent manner. The universal process of glycosylation occurs on the majority of microbe and host cells confirming the importance of appropriate CLR functioning. In this review, we provided an overview on the structural compositions of TM and soluble CLRs and we attempted to elucidate how CLRs form various conformations resulting in specific carbohydrate interactions.

Differences in structure and corresponding function among CLRs can be explained based on various receptor characteristics. The different signaling pathways triggered upon CLR-carbohydrate binding are crucial for the subsequent (immune) response. Upon activation, TM CLRs can act as endocytic receptors mediating the uptake of self or microbial antigens. In addition, the activation of both soluble and TM CLRs can modulate myeloid cell activation and affect inflammation and the induction of an adaptive immune response. Activator CLRs typically signal via Syk kinases, whereas inhibitory CLRs tend to recruit phosphatases. Interestingly, glycan ligand moiety and type are influencing signaling in for instance the DC-SIGN signalosome. Distinct DC-SIGN signaling pathways are induced upon binding by mannose or fucose-expressing microbes to the same Ca^{2+} binding site [40]. Apparently, the type of CLR functioning relies upon specific conformations formed via receptor-ligand binding.

Besides the ability of individual CLRs to function via distinct signaling pathways, several CLRs also express various isoforms. Different splicing variants and genomic polymorphisms have been determined, generating for instance MBP and DC-SIGN mRNA variants. These mRNA variants are reflected respectively in altered MBP serum levels and different sizes of the DC-SIGN extended neck regions[36, 63]. The presence of different CLR isoforms results in the ability of CLRs to recognize diverse branching, multivalency, and positioning of glycan residues on microbe and host cells.

CLRs express enhanced sensitivity upon the formation of multimer complexes. Besides homodimer, trimer and higher order complexes elucidated for TM and soluble CLRs, the presence of TM complexes with a heterologous character has been determined recently. Heterodimer formation of Dectin-2 and Dectin-3 on the cell surfaces enhances antifungal immunity[20]. Enhanced phagocytosis has been proven in rat cells upon the formation of a heterodimer complex between mincle and MCL [50]. Only few cases of heterodimer CLR formation are described so far. We suggest that there are many CLRs forming a wide variety of homo- and heterodimer complexes, facilitating diversity within the innate immune system to detect a broad spectrum of microbes. However, the lack of knowledge on individual CLR compositions and their corresponding ligands complicates the elucidation of heterologous CLR complexes. Besides homologous and heterologous complexes, CLRs are able to co-operate with other antigen sensing receptors to enhance immune responses. In conclusion, the enormous network of CLRs and other antigen sensing cells like Toll, RIG-1, and Nod-like receptors on the cell surface of host cells is forming a sophisticated network that is able to fine tune immune reactions by means of cooperation.

In this review we described the structure of both TM and soluble CLRs. The structures of these two groups of CLRs share high amount of overlap. However, the TM CLRs signal via intracellular signaling complexes whereas soluble CLRs tend to opsonize microbial agents followed by endocytosis and inflammatory responses resulting in adaptive immunity. Thus, despite their structural similarity, TM and soluble CLRs function in different ways. Interestingly, in literature TM

and soluble CLRs are solely described as two coexisting groups of CLRs. No data on interactions between individual CLRs of the two groups have been described. The possibility of microbes to be sensed with higher sensitivity by TM or soluble CLRs remains elusive. However, due to the structural overlap between TM and soluble CLRs it is conceivable that these two types of receptors compete with each other on an equal basis.

CLR targeting forms a promising tool for cell-specific drug delivery treatments and modulation of immunological function. Especially TM CLRs that have direct endocytic and signaling properties into the host cell form interesting targets for cell-specific drug delivery, for instance the development of new carbohydrate-based vaccination strategies. The different CLR structural conformations described in this review enhance sensitivity for microbial recognition in different ways. Understanding the advanced structural conformations of CLRs might lead to new approaches to study host-microbe interactions.

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