Chapter 13
The Ligands of C-Type Lectins

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Abstract In this chapter, a comprehensive overview of the known ligands for the C-type lectins (CTLs) is provided. Emphasis has been placed on the chemical structure of the glycans that bind to the different CTLs and the amount of structural variation (or overlap) that each CTL can tolerate. In this way, both the synthetic carbohydrate chemist and the immunologist can more readily gain insight into the existing structure-activity space for the CTL ligands and, ideally, see areas of synergy that will help identify and refine the ligands for these receptors.

Keywords C-type lectin • Receptor • Ligand • Pathogen • Immunity • Carbohydrates • Glycolipids

13.1 Introduction

There has been much interest in identifying the ligands that bind to and activate C-type lectins (CTLs) and in determining how this ligand-receptor binding modulates the immune response. As illustrated in Table 13.1, most CTL ligands (CTLLs) are from exogenous sources, although several examples of endogenous CTLLs can also be found. Most CTLLs contain a glycan motif; however, the breath of CTLLs is diverse and also includes proteins and oligonucleotides, as well as molecules whose structure is still to be determined. There are also several CTLs, such as CLEC12B, CLEC1-1, DCAL-1, DCAR and mDCAR1, for which there are no known ligands. As previous chapters in this book have focussed on the biochemical pathways and immunomodulatory activities of the CTLs, our focus herein has been to showcase the CTLLs and, where relevant, to highlight the degree of structural variation each CTL can tolerate. By doing so, we hope to equip the reader with a more readily digestible data set on the vast array of CTLLs and to encourage natural products or synthetic chemists to identify new, or further refine, ligands for these receptors.

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Table 13.1 C-type lectins and their ligands

Lectin	Structure	Origin	Lectin	Structure	Origin
Dectin-2	$Man_{7\text{-}9}GlcNAc_2$	Glycan array	SIGN-R1	Man, Fuc, Le^x, Le^y, SLe^x	Glycan array
	O-linked mannobiose-rich glycoproteins	*Malassezia*		Lacto-N-fucopentaose III (LNFP III)	Milk
	Mannose-capped LAM	*Mycobacterium*		Dextran	*Leuconostoc* spp.
MDL-1	Unidentified	Endogenous	LSECtin	GlcNAc, Fuc	Glycan array
BDCA-2	Non-sialylated complex-type glycans	Glycan array	Langerin	Man, Fuc, GlcNAc, $Man_9GlcNAc_2$	Glycan array
	Asialo-galactosyl-oligosaccharides	Chemical synthesis		Le^y, 6′-SO_4-Le^x	Glycan array
Mincle	Trehalose dimycolate (TDM)	*Mycobacterium*		Dextran sulphate	*Leuconostoc* spp.
	Trehalose diesters (TDE) and trehalose monoesters (TME)	Chemical synthesis		6-SO_4-Gal, 6-SO_4-GlcNAc, 6′-SO_4-LacNAc	Polyacrylamide (PAA)-conjugates
	α-Mannose and mannitol derivatives	*Malassezia*		Heparan sulphate (HS) and chondroitin sulphate (CS)	Porcine
MCL	TDM	*Mycobacterium*		Heparin (HEP)	Chemical synthesis
Dectin-1	T-cell ligand	Endogenous		Laminarin (β-1,6- and β-1,3-glucan)	*Laminaria digitata* (brown alga)
	Unidentified N-glycans	Tumour cells		Fucoidan (α-1,3/4-(2/3-SO_4)-fucan)	*Fucus vesiculosus*
	Linear and branched β-1,3-glucans	Plant and fungal cell walls		Galactan (β-1,4-)	Plant
CLEC-2	Rhodocytin	Snake venom		Mannan (β-1,4-)	*Saccharomyces cerevisiae*
	NeuAcα2-3Galα1-3(NeuAcα2-6) GalNAcα	Endogenous podoplanin		Dextran (α-1,3-branched α-1,6-glucans)	*Leuconostoc* spp.
	Fucoidan (α-1,3/4-(2/3-SO_4)-fucan)	*Fucus vesiculosus*		Zymosan (β-1,3-glucan)	*Saccharomyces cerevisiae*
DNGR-1	Filamentous actin (F-actin)	Necrotic cells		Mannose and β-glucans	Fungi

SIGN-R3	Dextran (α-1,3-branched α-1,6-glucans)	*Leuconostoc* spp.	MGL	α- and β-GalNAc (Tn antigen), LacdiNAc, Sialyl-Tn	Endogenous
	Zymosan (β-1,3-glucan)	*Saccharomyces cerevisiae*		MUC-1 and Muc-2	Endogenous
	High mannose	Glycan array	MGL-1	Gal, GalNAc, Le^x, Le^a	Glycan array
	Fucosylated glycans (Le^a and Le^b)	Glycan array		Gb5	Chemical synthesis
	ManLAM and LM	*Mycobacterium*	MGL-2	α- and β-GalNAc, Gal, Tn, TF, core 2,	Glycan array
	Unidentified	*Leishmania*, commensal fungi and bacteria		GalNAc	Endogenous
DCIR	HIV-1 glycoprotein 140 (gp140)	HIV	LOX-1	Modified lipoprotein	Chemically modified endogenous
	Sulfo-Le^a, Le^a, Le^b	Synthetic PAA conjugates		Advanced glycation end product (AGE)	Synthetic BSA Conjugates
	Manα1-3(Manα1-6)Man	Synthetic BSA conjugates		Polyinosinic acid (Poly I)	Synthetic
	Gal, GalNAc, Glc and GlcNAc	Synthetic BSA conjugates		Carrageenan (type III Kappa)	Red algae
DCIR-2	N-glycan with bisecting GlcNAc	Chemical synthesis		Phosphatidylserine, phosphatidylinositol	Liposomes
MICL	Mono sodium urate (MSU) crystals	Synthetic		Phosphatidic acid, cardiolipin, phosphatidylglycerol	Liposomes
LY49Q	MHC-I	Endogenous	MR	ManLAM	*Mycobacterium*
DC-SIGN	Le^x, Le^y, LDNF	*Schistosoma mansoni*		Mannan	*Saccharomyces cerevisiae*
	ManLAM, PIM, α-glucan	*Mycobacterium*		Fuc, Man	Neoglycoproteins
	$Man_9GlcNAc_2$	Glycan array		3- and 4-SO_4-GalNAc	Chemical synthesis
	Mannan	*Saccharomyces cerevisiae*		Chondroitin sulphates A and B	Bovine and porcine
	TAG-72, semen clusterin	Endogenous		SO_4-Le^x, SO_4-Le^a	Chemical synthesis
	HIV-1 glycoprotein 120 (gp120)	HIV		High-mannose-containing glycoproteins	Endogenous
L-SIGN	$Man_9GlcNAc_2$	Chemical synthesis	DEC-205	CpG oligonucleotides	Chemical synthesis

13.2 C-Type Lectins Containing ITAM-Like Signalling Motifs

13.2.1 Dectin-2

Dectin-2, otherwise known as CLEC6A, is an FcRγ-coupled receptor found on macrophages (Mφs), monocytes and several subsets of dendritic cells (DCs) (Sancho and Reis e Sousa 2012). Binding assays have demonstrated that the extracellular portion of Dectin-2 can recognise the hyphal portions of *Candida albicans*, *Microsporum audouinii* and *Trichophyton rubrum* (Sato et al. 2006). In addition to fungal species, Dectin-2 has been shown to recognise *Schistosoma mansoni* egg antigens (Ritter et al. 2010). Although the exact ligand structure for Dectin-2 is not well defined, carbohydrate binding studies have revealed that Dectin-2 recognises high-mannose structures such as $Man_9GlcNAc_2$ (Fig. 13.1) (McGreal et al. 2006). Here, Dectin-2 was screened against 109 synthetic carbohydrates, and although Dectin-2 displayed the highest specificity for $Man_9GlcNAc_2$, it also recognised $Man_8GlcNAc_2$ and $Man_7GlcNAc_2$. Dectin-2 has also been shown to bind α-mannans of fungal cell walls (Saijo et al. 2010).

Dectin-2, in cooperation with Mincle, has been shown to recognise the pathogenic fungus *Malassezia*. Using solvent-based fractionation, it was determined that Dectin-2 recognised the hydrophilic components of *Malassezia*, and with the aid of mass spectrometry and NMR analysis, an *O*-linked mannobiose-rich glycoprotein was determined to be the Dectin-2 ligand (Ishikawa et al. 2013). The mannose-capped lipoarabinomannan LAM from mycobacterial species has also been identified as a ligand for Dectin-2 (Yonekawa et al. 2014). Moreover, Dectin-2 recognises a ligand on $CD4^+CD25^+$ T cells; however, the exact ligand structure is unknown (Aragane et al. 2003).

13.2.2 MDL-1

MDL-1 (myeloid DAP12-associating lectin-1 or CLEC5A) is a CTL expressed on the cell surface of monocytes, Mφs and osteoclasts (Sancho and Reis e Sousa 2012). MDL-1 has been shown to bind the dengue virion (DV), resulting in DAP12 phosphorylation and cytokine production (Chen et al. 2008). If, on the other hand, the DV-MDL-1 interaction is blocked with monoclonal antibodies, symptoms associated with DV infection, such as plasma leakage and vital organ haemorrhaging, were reduced in a murine model. In addition to the dengue virion, MDL-1 also directly interacts with the Japanese encephalitis virus and induces cytokine production by Mφs (Chen et al. 2012). MDL-1 knockout or blocking with an MDL-1 antibody was shown to reduce the symptoms of arthritis, which indicates that there may be an unidentified self-ligand for MDL-1 (Joyce-Shaikh et al. 2010). The same authors later proposed that galectin-9 was a ligand for MDL-1 and

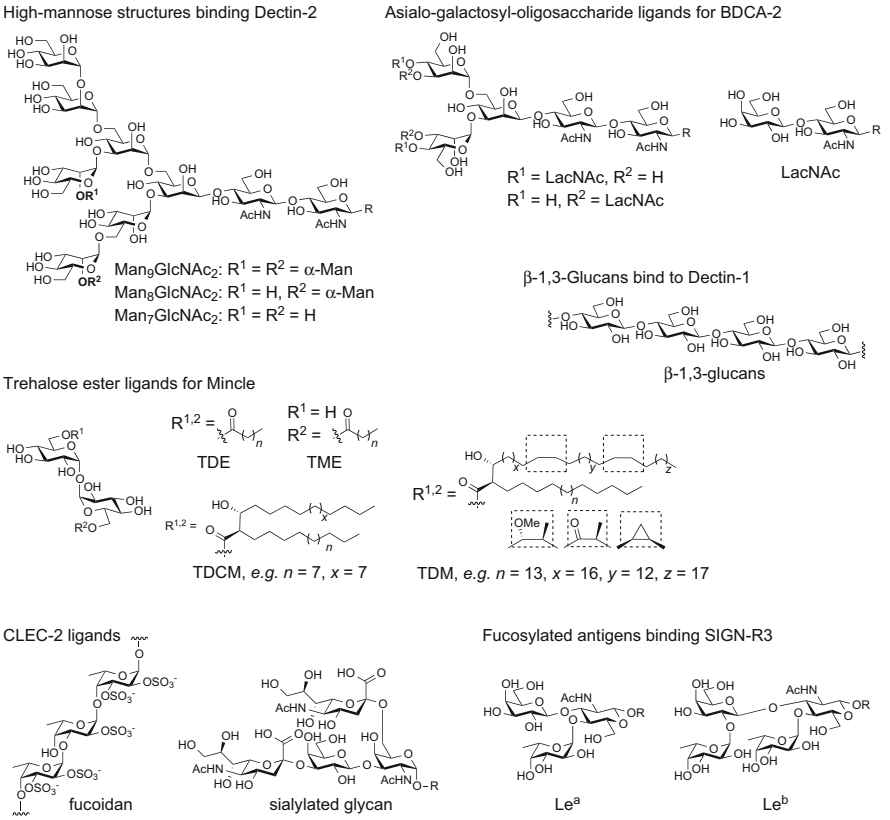

Fig. 13.1 Representative ligands for ITAM-like and Hem-ITAM-like receptors

demonstrated that treatment with galectin-9 intensified disease in a murine model of arthritis (Joyce-Shaikh et al. 2014).

13.2.3 BDCA-2

Blood dendritic cell antigen 2 (BDCA-2, also known as CLEC4C or CD303) belongs to the group II ITAM-coupled family of myeloid CLRs. BDCA-2 expression is restricted to human plasmacytoid DCs (Dzionek et al. 2001), and the targeting of antigens to this receptor has been suggested to be a promising strategy for inducing antigen-specific tolerance. BDCA-2 was first shown to bind the HIV protein, gp120, leading to the inhibition of toll-like receptor (TLR)-9-mediated activation and interferon (IFN)-γ secretion in plasmacytoid DCs (Martinelli et al. 2007), while more recently it was determined that BDCA-2 recognises complex-type sugars that have lost their terminal sialic residues (Fig. 13.1), such as

Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)-Manβ1-4GlcNAcβ1-4GlcNAcβ and Galβ1-3GlcNAcβ1-2Manα1-3(Galβ1-3GlcNAcβ1-2Manα1-6)-Manβ1-4GlcNAcβ1-4GlcNAcβ (Riboldi et al. 2011). The recognition of complex galactose-terminated glycans by BDCA-2 has been suggested to be a mechanism that tumour cells or invading pathogens use to downregulate IFN-γ production and immune surveillance.

13.2.4 Mincle

Macrophage-inducible C-type lectin (Mincle, CLEC4e or CLECf9) is a group II FcRγ-coupled receptor that is expressed in low levels on Mφs and DCs (Sancho and e Sousa 2012). In a ligand-binding study that employed an NFAT-GFP reporter cell line and Mincle-deficient mice, it was demonstrated that trehalose dimycolate (TDM), the most abundant glycolipid in the mycobacterial cell wall, is a ligand for Mincle (Ishikawa et al. 2009). TDMs consist of a trehalose disaccharide core bound to two mycolic acid chains, which can be of varying structural complexity (Fig. 13.1). Additionally, both human and murine Mincle have been shown to recognise the yeast species *Candida albicans* (Bugarcic et al. 2008; Wells et al. 2008) and the fungal species *Malassezia* (Yamasaki et al. 2009). In *Malassezia* fungal species, specific mannitol-containing ligands were identified as direct ligands for Mincle (Ishikawa et al. 2013). Mincle has also been shown to sense dead cells through a protein component of small nuclear ribonucleoprotein (SAP30) (Yamasaki et al. 2008).

Structure-activity relationship studies have since confirmed that many simple TDM synthetic analogues also bind Mincle. Specifically, long-chain trehalose diesters (TDEs), including trehalose dibehenate (TDB), have been found to lead to the robust activation of Mφs (Schoenen et al. 2010; Khan et al. 2011), while more recently it has been demonstrated that trehalose esters with only one lipid chain (trehalose monoesters, TMEs) can also bind and activate Mincle (Stocker et al. 2014). Moreover, functionalised trehalose glycolipids, including those containing fluorescent reporter groups or photoaffinity probes, have also been shown to bind and activate Mincle (Khan et al. 2013; Kodar et al. 2015). In addition to trehalose diesters, another mycobacterial immunostimulatory lipid, glycerol monomycolate, has been shown to activate human but not mouse Mincle (Hattori et al. 2014). Corynomycolic esters of trehalose (TDCM) were also found to be potent activators of both mouse and human Mincle, while 2-*S*-corynomycolic esters of glycerol were found to activate human but not mouse Mincle (van der Peet et al. 2015).

13.2.5 MCL

Macrophage C-type lectin (MCL or CLEC4d) is another FcRγ-coupled receptor that is constitutively expressed on myeloid cells (Sancho and Reis e Sousa 2012). MCL is thought to arise via a gene duplication of Mincle, and while MCL contains a calcium coordination site, it does not retain the exact EPN motif of Mincle (Sancho and Reis e Sousa 2012). The exact ligand structure of MCL is not well defined; however, MCL has been shown to bind TDM (Miyake et al. 2013; Furukawa et al. 2013).

13.3 C-Type Lectins Containing Hem-ITAM-Like Signalling Motifs

13.3.1 Dectin-1

Dectin-1 (also known as CLEC7a) was the first non-toll-like receptor shown to mediate its own intracellular signalling (Sancho and Reis e Sousa 2012). The receptor was originally found to recognise an endogenous T-cell ligand of unknown structure (Ariizumi et al. 2000) and has since been shown to bind other endogenous ligands including undefined N-glycans on the surface of tumour cells, which leads to tumour destruction by natural killer (NK) cells (Chiba et al. 2014), and vimentin (a type III intermediate filament protein) (Thiagarajan et al. 2013). An unknown ligand in mycobacteria also binds Dectin-1 (Yadav and Schorey 2006; Rothfuchs et al. 2007).

The most insight into the structure of the Dectin-1 ligands, however, concerns the ability of the receptor to bind glucans, particularly β-1,3-glucans (Fig. 13.1), which are found in the cell wall of plants and fungi (Brown and Gordon 2001; Sancho and Reis e Sousa 2012). Microarray studies suggest that ten to eleven β-1,3-linked glucose oligomers are required for optimal Dectin-1 binding, and while β-1,6-glucans of comparable length do not bind (Palma et al. 2006), branched β-1,3-glucans do bind to the receptor (Palma et al. 2006; Adams et al. 2008). More recent studies have demonstrated that smaller glucan mimetics can also bind Dectin-1, as evidenced by studies using synthetic β-glu6 containing an α-(1→3)-linked bond (Li et al. 2013) and di- and trimeric hydroxylamine-based mimetics (Ferry et al. 2014). In the latter study, the binding of the small oligosaccharide fragments was attributed to the increased hydrophobic interaction between the α-face of the di- or trisaccharide and the aromatic side chains of Trp 221 ad His 223 in the binding site of Dectin-1. A distinction has also been made between the binding of soluble and particulate glucans. Different downstream Dectin-1 signalling occurs when myeloid cells come into contract with particulate β-glucan, and in particular, that prolonged Dectin-1 signalling occurs when myeloid cells come into contact with large β-glucan particles (Sancho and Reis e Sousa 2012). A few

polysaccharides can also interfere with the binding of β-glucans to Dectin-1, thereby thwarting the host immune response. These include chitin-like components found on sclerotic cells in murine models of chromoblastomycosis (Dong et al. 2014) and α-(1,3)-glucan, which is a cell wall constituent of most fungal respiratory pathogens (Rappleye et al. 2007).

13.3.2 CLEC-2

Like Dectin-1, CLEC-2 (also known as CLEC-1B or CLEC-2B) also belongs to Group V of the non-calcium-dependent CTLs. CLEC-2 was discovered with CLEC-1 in a bioinformatics screen for NK receptors, and while mRNA for CLEC-2 has been found in bone marrow cells, DCs, monocytes, granulocytes and some NK cell populations, most studies have focussed on the role of CLEC-2 on platelets (Plato et al. 2013). Several CLEC-2 ligands have been identified, including rhodocytin (aggretin), which is an exogenous multimeric protein found in snake venom that leads to platelet activation and aggregation and subsequent coagulation of the blood (Suzuki-Inoue et al. 2011). Soon thereafter, the endogenous protein podoplanin was found to be a ligand for CLEC-2 (Suzuki-Inoue et al. 2007). Podoplanin is found in multiple cell types such as lymphatic endothelial cells, type I lung alveolar cells and in some cancer cells and consists of the sialylated glycan NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc (Fig. 13.1) conjugated via an α-linkage to Thr52 in the platelet aggregation-stimulating domain (Kaneko et al. 2007). Both the disialyl core and the stereostructure of the protein were found to be critical for CLEC-2 binding, as evidenced by the observation that CLEC-2-Fc terminal deletion mutants and human podoplanin glycopeptides containing truncated glycans were unable to bind the receptor (Kato et al. 2008). Fucoidan (Fig. 13.1), which is a sulphated polysaccharide from *Fucus vesiculosus*, is an agonist for CLEC-2 (Manne et al. 2013), and HIV is also thought to be recognised by CLEC-2, although it is proposed that this recognition is due to the incorporation of a host protein into HIV during budding (Suzuki-Inoue et al. 2011). Finally, a series of synthetically prepared nucleic acid CLEC-2 ligands (aptamers) were identified using the systematic evolution of ligands by exponential enrichment (SELEX) methodology (Layzer et al. 2010).

13.3.3 DNGR-1

The DC, NK lectin group receptor-1 (DNGR-1), also known as CLEC9A, is expressed on specific subsets of DCs (Sancho and Reis e Sousa 2012). The receptor recognises filamentous actin (F-actin), which is exposed on the surface of necrotic cells and thus serves as an evolutionarily conserved damage-associated molecular pattern (Ahrens et al. 2012; Zhang et al. 2012). The binding of F-actin to DNGR-1

does not lead to pro-inflammatory responses, but, rather, signalling from the receptor is required for antigen cross-presentation and effective immunity (Plato et al. 2013). The ability of DNGR-1 to promote antigen cross-presentation has seen interest in the development of a peptide-conjugate vaccine via use of an anti-DNGR-1 antibody conjugated to the tumour-associated glycoprotein antigen, MUC1 (Picco et al. 2014).

13.3.4 SIGN-R3

Mouse SIGN-R3 (CD209d) is a receptor with endocytic activity and is part of a cluster of mouse SIGN-R genes that are highly homologous to human DC-SIGN; although unlike human DC-SIGN, mouse SIGN-R3 signals via a Syk-dependent pathway (Sancho and Reis e Sousa 2012). Ligand studies using transfected non-macrophage cell lines demonstrated that SIGN-R3 endocytosed dextran of 40 kDa or greater and zymosan (Takahara et al. 2004). A comprehensive glycan array analysis further refined the SIGN-R3 ligands and demonstrated that SIGN-R3 preferentially binds to high-mannose glycans and fucosylated glycans, particularly Lewis^a (Le^a) and Le^b antigens (Fig. 13.1) (Galustian et al. 2004), with subsequent array studies supporting these findings (Powlesland et al. 2006). SIGN-R3 has been found to contribute to early host resistance to *M. tuberculosis* infection with mycobaterial ManLAM and LM, but not AraLAM, binding and activating the receptor (Tanne et al. 2009). More recently, SIGN-R3 has been shown to play a role in leishmaniasis infection (Lefèvre et al. 2013) and to recognise ligands in commensal fungi and bacteria thereby potentially mediating colitis (Eriksson et al. 2013; Lightfoot et al. 2015); however, in each study, the specific ligand was not identified.

13.4 C-Type Lectins Containing ITIM-Like Signalling Motifs

13.4.1 DCIR

The C-type lectin dendritic cell immunoreceptor (DCIR) is an ITIM-coupled receptor. Human DCIR (CLEC4a) is expressed in monocytes, Mφs, granulocytes, B cells and DCs (Sancho and Reis e Sousa 2012). It has been demonstrated that DCIR can bind HIV-1 (Lambert et al. 2008), and while the specific ligand was not identified during this study, later work demonstrated that DCIR interacts with Le^b and Le^a (Fig. 13.2), mannotriose and sulfo-Le^a (Fig. 13.2) as well as the HIV-1-type glycoprotein, gp140 (Bloem et al. 2014). In a competitive binding study that compared the binding characteristics of several CTLs, DCIR was found to bind

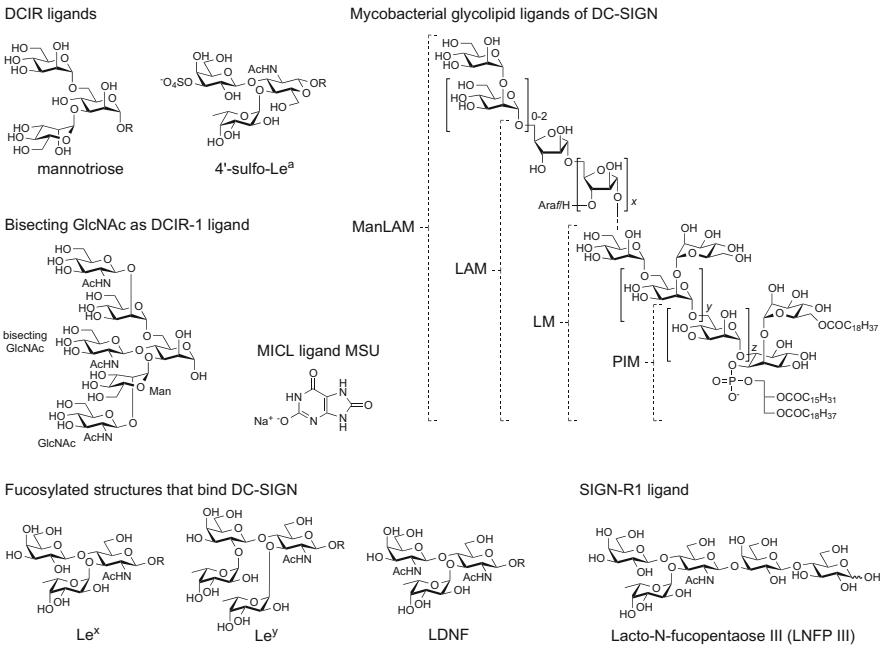

Fig. 13.2 Representative ligands for ITIM-like and ITAM-ITIM-independent CTLs

mannose- and fucose-based ligands as well as thio-linked Gal-, GalNAc-, Glc- and GlcNAc-BSA (Lee et al. 2011). In an additional study, it was demonstrated that purified DCIR could bind the glycan structures Le^b and Man_3; however, this binding was not detected when the DCIR was expressed on the cell surface (Bloem et al. 2013).

13.4.2 DCIR-1 and DCIR-2

Four DCIR homologues have been identified in mice (DCIR-1-4); however, only DCIR-1 and DCIR-2 contain an ITIM sequence. DCIR-1 is expressed in B cells, monocytes, Mφs and DCs; however, very little is known about its ligands (Sancho and Reis e Sousa 2012). DCIR-2, on the other hand, is expressed on DCs and has been found to specifically bind *N*-glycans that incorporate bisecting *N*-acetyl-glucosamine (a β-GlcNAc moiety attached to the N-glycan β-mannose 4-position) (Nagae et al. 2013). Here, the authors noted that DCIR-2 primarily recognises two residues including the GlcNAcβ1-2Manα1-3- and bisecting GlcNAc residues (Fig. 13.2).

13.4.3 MICL

Human myeloid inhibitory C-type lectin (MICL also known as DCAL-2, KLRL-1, CLL-1 and CLEC-12a) is an ITIM-coupled receptor expressed in granulocytes, monocytes, Mφs and DCs (Sancho and Reis e Sousa 2012). Murine MICL on the other hand is expressed in myeloid cells, B cells, $CD8^+$ T cells and bone marrow NK cells. Using flow cytometry and an Fc-mMICL fusion protein, MICL was found to bind several endogenous ligands from the heart, lung, liver, spleen and kidney (Pyz et al. 2008). More recently, MICL was determined to be a receptor for dead cells derived from 293T cells or thymocytes (Neumann et al. 2014). Here, ligand-binding studies demonstrated that both human and mouse MICL can recognise uric acid crystals (monosodium urate, Fig. 13.2), which are well-known cell death danger signals (Neumann et al. 2014).

13.4.4 LY49Q

Mouse Ly49Q (Klral7) is an inhibitory receptor that is expressed in $Ly6C/G^+$ myeloid precursors, immature monocytes and plasmacytoid DCs (Sancho and Reis e Sousa 2012). Reporter cell analysis was employed to demonstrate that H-2^b-derived tumour cells contain a high-affinity MHC-Ia-like ligand for LY49Q (Tai et al. 2007). In a subsequent study, LY49Q was identified as a direct receptor for MHC-I in mice (Scarpellino et al. 2007).

13.5 ITAM-ITIM-Independent C-Type Lectins

13.5.1 DC-SIGN

Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), also known as CD209, is a CTL involved in DC-T-cell contact. DC-SIGN has a single carbohydrate recognition domain (CRD) and binds branched D-mannose and L-fucose motifs common on pathogen surfaces (Sancho and Reis e Sousa 2012), with clustering of the lectins resulting in the formation of tetramers to enhance ligand binding (Mitchell et al. 2001). High-mannose oligosaccharide ligands include mannan, mannosylated lipoarabinomannan (ManLAM, Fig. 13.2) (Maeda et al. 2003), phosphatidyl-*myo*-inositol mannosides (PIM, Fig. 13.2) (Driessen et al. 2009) and $Man_9GlcNAc_2$ (Fig. 13.1), whereby the latter binds DC-SIGN with 130 times higher affinity than mannose (Mitchell et al. 2001). DC-SIGN has a higher affinity for L-fucose than mannose and recognises branched fucosylated structures with terminal galactose residues, such as the Lewis antigens (Coombs et al. 2005), in particular Le^x and possibly LDNF (Fig. 13.2) (van Die

et al. 2003). DC-SIGN binds *Helicobacter pylori* (Miszczyk et al. 2012) and *S. mansoni* (Meyer et al. 2005) through the Le^x and Le^y antigens, while *Mycobacterium tuberculosis* is recognised via ManLAM (Maeda et al. 2003), PIM (Driessen et al. 2009) and α-glucan (Geurtsen et al. 2009), as well as further unidentified ligands (Ehlers 2010). Although this implies broad specificity for branched sugars, DC-SIGN discriminates between ligands through secondary binding sites and the α/β-linkage of adjacent saccharides (van Die et al. 2003). The signalling pathways induced by DC-SIGN are dependent on the nature of the ligand, leading to endocytosis or modulation of gene expression (Sancho and Reis e Sousa 2012). Endogenous ligands include tumour-associated glycoprotein-72 (TAG-72) (Laskarin et al. 2011) and semen clusterin (Sabatte et al. 2011), while DC-SIGN acts as a receptor for HIV through binding to the HIV-1 gp120 envelope protein (Curtis et al. 1992).

13.5.2 L-SIGN

L-SIGN or DC-SIGNR (also known as CD299, CD209L and CLEC4M) is a type II transmembrane C-type lectin receptor with 77 % sequence homology to DC-SIGN. In contrast to DC-SIGN which is expressed on DCs, L-SIGN is highly expressed on liver sinusoidal cells, endothelial vascular cells and in the lymph nodes. Like DC-SIGN, L-SIGN has high-affinity binding to a variety of ligands, including ICAM-3, HIV gp120-binding protein, simian immunodeficiency virus, Ebola virus, hepatitis C virus and respiratory syncytial virus. The CRD of L-SIGN binds the Man9GlcNAc2 oligosaccharide (Fig. 13.1) 17-fold more tightly than mannose, and its affinity for a glycopeptide bearing two $Man_9GlcNAc_2$ oligosaccharides is further increased by fivefold to 25-fold. These results indicate that the CRDs contain extended or secondary oligosaccharide binding sites. When the CRDs are clustered in the tetrameric extracellular domain, their arrangement provides a means of amplifying specificity for multiple glycans on host molecules targeted by DC-SIGN and L-SIGN (Mitchell et al. 2001).

13.5.3 SIGN-R1

The mouse CTL-specific ICAM-3 grabbing nonintegrin-related 1 (SIGN-R1, CD209b) is a homologue of hDC-SIGN and is expressed on a limited subset of Mφs and endothelial cells with a cell-specific expression similar to that of hL-SIGN. SIGN-R1 binds mannose- and fucose-containing ligands and Lewis blood antigens, thereby mirroring the specificity of hDC-SIGN and hL-SIGN, but in addition, SIGN-R1 also interacts with sialylated Le^x (Galustian et al. 2004; Koppel et al. 2005). Lacto-N-fucopentaose III (LNFPIII, Fig. 13.2) binds to the surface of cells transfected with SIGN-R1, and binding of LNFPIII-NGC to SIGN-R1 has been demonstrated by ELISA (Srivastava et al. 2014). SIGN-R1 binds

zymosan, a glucan with repeating glucose units connected by β-1,3-glycosidic linkages, and the capsular polysaccharide of *Streptococcus pneumoniae*, and while SIGN-R1-Fc did not interact with dextran, which contains a combination of α-1,3- and α-1,6-glucose linkages, cellular-expressed SIGN-R1 does interact with dextran, as demonstrated by several groups (Geijtenbeek et al. 2002; Kang et al. 2003).

13.5.4 LSECtin

Liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin, CLEC4G) is a ~40 kDa type II integral membrane protein with a single C-type lectin-like domain, closest in homology to DC-SIGNR, DC-SIGN and CD23 (Liu et al. 2004). LSECtin functions as an attachment factor for Ebola virus and SARS, but it does not bind HIV or hepatitis C virus (Gramberg et al. 2005). LSECtin exhibits ligand-induced internalisation, and its sugar recognition specificity differs from that of DC-SIGN, as sugar-binding studies indicate that LSECtin specifically recognises *N*-acetyl-glucosamine (Dominguez-Soto et al. 2007) and L-fucose (Liu et al. 2004), whereas no LSECtin binding to mannan, *N*-acetyl-galactosamine and galactose were observed. The presence of LSECtin on myeloid cells should therefore contribute to expanding their antigen-capture and pathogen-recognition capabilities.

13.5.5 Langerin

Langerin (CLEC4K, CD207) is a type II transmembrane receptor with an extracellular region consisting of a neck and a C-terminal C-type CRD and is highly expressed on Langerhans cells, $CD103^+$ DCs and splenic $CD8^+$ DCs. Langerin recognises a wide array of carbohydrates including mannose, fucose and GlcNAc structures and especially $Man_9GlcNAc_2$ (Stambach et al. 2003). Langerin also recognises the difucosylated oligosaccharide Le^y (Holla et al. 2011) and Le^x-type sequences that are sulphated at the 6-position of the outer galactose (6′-sulfo-3′-SLe^x, Fig. 13.3). This specificity is unique among the CTLs and contrasts markedly with the selectins which bind the analogous Le^x structures that are sulphated at the 3-position of the galactose. Of the sulphated saccharides, Langerin has also been shown to bind dextran-sulphate (Galustian et al. 2004) and 6-sulphated GlcNAc and especially 6′-sulfo-LacNAc (Tateno et al. 2010), whereas no binding was observed for either its positional isomer, 6-sulfo-LacNAc, or its unsulphated form. Langerin also binds to glioblastoma tissues via Gal-6-sulphated glycans (Tateno et al. 2010). Taken together, this suggests that the sulphate at C-6 of the non-reducing end sugar might be important for Langerin recognition.

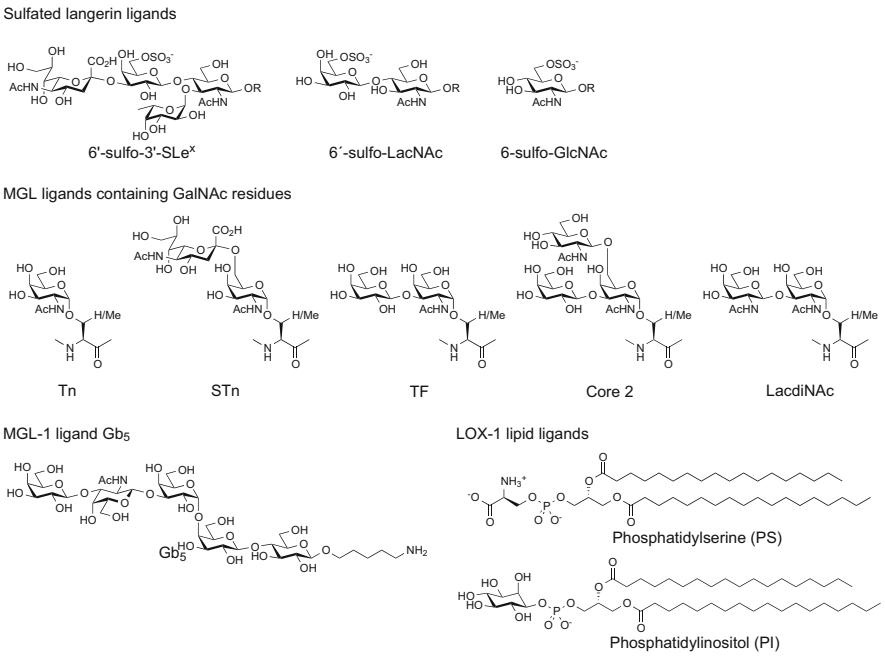

Fig. 13.3 Representative ligands for ITAM-ITIM-independent CTLs

Other studies have shown that glycosaminoglycans such as heparan sulphate (HS) and chondroitin sulphate (CS) (preferentially 4-O-sulphated) interact with Langerin through a Ca^{2+}-independent glycosaminoglycan (GAG)-specific binding site (Chabrol et al. 2012). Depending on ligand size, there are two binding modes for HS/heparin (HEP) oligosaccharides: a Ca^{2+}-dependent mode for small HEP trisaccharides and binding at a positively charged groove at the interface of two CRDs and the neck domain for large (6 kDa) HEP oligosaccharides (Muñoz-García et al. 2015). Further, polysaccharides found to bind Fc-Langerin include laminarin, fucoidan, galactan and α-mannan (Hsu et al. 2009), and langerin was shown to bind to high molecular weight dextran (250–2,000 kDa) and zymosan, although binding was inhibited by mannan (Takahara et al. 2004).

Langerin binds to a variety of microorganisms: langerin is a receptor for *Yersinia pestis* phagocytosis and promotes dissemination (Yang et al. 2015), and carbohydrate-dependent binding of langerin to a cell wall glycoprotein of *Mycobacterium leprae* has been observed (Kim et al. 2015). Langerin binds HIV-1, which prevents transmission (De Witte et al. 2007). Also *Candida* and *Saccharomyces* species and *Malassezia furfur* are recognised by langerin, but very weak binding was observed to *Cryptococcus gattii* and *Cryptococcus neoformans*. No binding was observed for the Gram-positive bacteria *Staphylococcus aureus* or Gram-negative *Escherichia coli* and *Salmonella typhimurium* (Takahara et al. 2004). Notably, Langerin has been identified as the primary fungal receptor

on Langerhans cells (LCs), since the interaction of LCs with fungi was blocked by antibodies against Langerin. Langerin recognises both mannose and β-glucans present on fungal cell walls and appears to be an important fungal pathogen receptor on human LCs that recognises pathogenic and commensal fungi (De Jong et al. 2010). Interestingly, common polymorphisms in human langerin change the receptor specificity for glycan ligands (Feinberg et al. 2013).

13.5.6 MGL

Macrophage galactose-type lectin (MGL, CLEC-10A, CD301, DC-ASGPR), a type II transmembrane receptor, is expressed on immature and tolerogenic DCs, Mφs, dermal $CD1a^+$ DCs and blood $CD1c^+$ myeloid DCs. MGL specifically recognises α- and β-linked GalNAc residues (Suzuki et al. 1996), including the Tn antigen and LacdiNAc (Fig. 13.3) and 6-substituted GalNAc derivatives such as the sialyl-Tn antigen (Mortezai et al. 2013). In addition, MGL binds to CD45 on effector T cells and interacts with lymphatic endothelial cells through an unknown ligand. Of the self-antigens, MGL binds to the GalNAc moieties on the tumour-derived MUC1 and MUC2 glycoproteins (Iida et al. 1999). Pathogenic organisms that engage with MGL include *Neisseria gonorrhoeae*, *Campylobacter jejuni*, Ebola virus, *Schistosoma mansoni* (Van Vliet et al. 2005) and *Trichuris suis* (Van Kooyk et al. 2015).

13.5.7 MGL-1

The mouse CTL MGL-1 (CD301a, CLEC-10a) is one of the two hMGL orthologues with distinct carbohydrate recognition. Early studies demonstrated that mMGL-1 recognises galactose-related structures such as Le^x (Tsuiji et al. 2002). Using a glycan array, murine MGL-1 was found to be highly specific for Le^x and Le^a structures. The generation of MGL-1-Fc proteins allowed the identification of high endothelial venules as ligands in the lymph nodes (Singh et al. 2009), while the incubation of arrays with an MGL-1-hFc fusion protein showed up to tenfold increased binding to multiantennary N-glycans displaying Le^x structures compared to monovalent Le^x trisaccharide (Eriksson et al. 2014). In another glycan array, MGL-1 was found to bind to a range of terminal galactose and GalNAc glycans, which is consistent with the known galactose-binding motif QPD in the CRD of MGL-1. In these studies, MGL-1-Fc was found to bind the stage-specific tumour antigen Gb5 (Fig. 13.3), which suggests an association of this interaction with tumour progression (Maglinao et al. 2014).

MGL-1 also binds *Trypanosoma cruzi*, presumably through surface-expressed galactose moieties (Vázquez et al. 2014), and triggers phosphorylation of Raf-1 in response to products excreted/secreted by the helminth parasite *Taenia crassiceps*,

thereby being involved in the induction of Th2 responses against the parasite (Terrazas et al. 2013). MGL-1 acts as an attachment and entry receptor for influenza virus, independent of sialic acid expression (Upham et al. 2010; Ng et al. 2014). Recombinant MGL-1 was found to bind both *Streptococcus* sp. and *Lactobacillus* sp. among commensal bacteria isolated from mesenteric lymph nodes of mice treated with dextran sulphate sodium salt (DSS) (Saba et al. 2009).

13.5.8 MGL-2

MGL-2 (CD301b) recognises carbohydrates containing GalNAc, which is similar to the carbohydrate specificity of human MGL (Tsuiji et al. 2002). Using a glycan array, MGL-2 was found to recognise GalNAc and galactose, including the O-linked Tn- and TF-antigens and core 2 O-GalNAc glycans (Fig. 13.3). Strikingly, MGL-2 interacted strongly with adenocarcinoma cells, suggesting a potential role in tumour immunity (Singh et al. 2009). MGL-2 specifically binds tumour-associated GalNAc, and modification of an antigen with GalNAc targeted the antigen specifically to the MGL-2 on bone marrow-derived (BM) DCs and splenic DCs and promoted antigen internalisation in DCs and presentation to CD4 T cells, as well as differentiation of IFN-γ producing CD4 T cells (Singh et al. 2011). Mice infected with the natural rodent hookworm pathogen *Nippostrongylus brasiliensis* required MGL-2^+ DCs for efficient Th2 development, but these cells were dispensable for T follicular helper or B-cell responses, as MGL-2^+ DC-depleted animals showed normal levels of IgG1 and IgE antibodies (Kumamoto et al. 2013).

13.5.9 LOX-1

Lectin-like oxidised low-density lipoprotein (ox-LDL) receptor-1 (LOX-1, CLEC8A) has been identified as the receptor for five diverse ligand classes (Chen and Du 2007). The first class is modified lipoproteins including ox-LDL (Sawamura et al. 1997), hypochlorite-modified high-density lipoprotein (HOCl-HDL) (Marsche et al. 2001), carbamylated LDL (Apostolov et al. 2009), electronegative LDL (Lu et al. 2009), apolipoprotein B (Gillotte et al. 2000; Okamura et al. 2013, and advanced glycation end product (AGE) proteins (Jono et al. 2002). Native LDL is not recognised by LOX-1 (Yoshimoto et al. 2011), while some studies suggest that acetylated LDL is recognised (Shi et al. 2001), and others indicate it is not (Moriwaki et al. 1998). The second group of ligands are polyanionic structures including polyinosinic acid and carrageenan (Moriwaki et al. 1998). Anionic phospholipids are also recognised, including the cellular ligands phosphatidylserine (PS) and phosphatidylinositol (PI) (Fig. 13.3) (Oka et al. 1998), which are expressed on apoptotic and aged cells. Cellular ligands comprise the fourth group; however, the exact molecules recognised by LOX-1 on these cells are less

well defined. Apoptotic cells (Oka et al. 1998), platelets (Kakutani et al. 2000) and both Gram-negative bacteria such as *Escherichia coli* and Gram-positive bacteria such as *Staphylococcus aureus* are recognised (Shimaoka et al. 2001). Finally, other macromolecules that act as LOX-1 ligands include bile salt-dependent lipase (Chen and Du 2007), heats-hock proteins (Murshid et al. 2011) and C-reactive proteins (Shih et al. 2009).

13.5.10 Mannose Receptor

The mannose receptor (MR), also known as CD-206, is a 175-kDa transmembrane C-type lectin widely expressed on tissue Mφs and DCs (Martinez-Pomares 2012). MR binds branched high-mannose-containing motifs, such as ManLAM (Kang et al. 2005) and mannan (Taylor et al. 1992), and while MR preferentially binds branched α-linked oligomannoses, its specificity is not limited to D-mannose-containing glycoconjugates. Affinity binding competition studies demonstrate that MR binds to glycoconjugates with the following specificity: L-Fuc = D-Man > D-GlcNac ≈ D-Glc > D-Xyl >> D-Gal = L-Ara = D-Fuc, while galactose and GalNAc do not bind (Shepherd et al. 1981). Ligand binding is mediated by eight tandem CRDs, and while CRDs 1–3 have weak ligand binding, CRD-4 is able to elicit monosaccharide binding, and CRDs 4–8 are necessary for the binding of complex glycans (Mullin et al. 1994; Taylor et al. 1992). MR has a secondary lectin binding site located at the cysteine-rich N-terminus of the protein, and this mediates binding to sulphated sugar residues (Leteux et al. 2000), with special affinity for GalNAc residues sulphated at the 3- and 4-positions, including chondroitin sulphates A and B and sulphated Le^x and Le^a.

Given the breath of glycans to which MR binds, the receptor is thus able to recognise a variety of pathogens including viruses, fungi, bacteria and helminths, resulting in their phagocytosis, and many specific allergens including Ara h 1 (peanut), Bla g2 (cockroach), Can f 1 (dog), Der p 1 (mite), Der p 2 (mite) and Fel d 1 (cat) (Martinez-Pomares 2012). MR also acts as a molecular scavenger, and indeed the receptor was initially identified due to its ability to clear high-mannose-containing glycoproteins, such as lysosomal enzymes, from blood (Stahl et al. 1976; Martinez-Pomares 2012). Other endogenous ligands include salivary amylase, tissue plasminogen activator, thyroglobulin and serum secretory phospholipase A2-IIA (Martinez-Pomares 2012).

13.5.11 DEC-205

Mouse DEC-205 (CD205), a CTL that is highly expressed on $CD8\alpha^+$ DCs and to a lesser degree on macrophages, T cells, B cells, and granulocytes, recognises plasminogen activator (PLA) expressing bacteria such as Yersinia pestis and

Escherichia coli but not the PLA-negative controls (Sancho and Reis e Sousa 2012; Zhang et al. 2008). Both murine and human DEC-205 (which is widely expressed) act as receptors for dying cells (Shrimpton et al. 2009).

13.6 Summary

As evidenced above, the repertoire of CTLs and their associated ligands is immense. Some CTLs have been studied for many years, and, accordingly, their associated ligands are, by and large, well defined; however, for other CTLs, and especially those that appear to accommodate a vast array of ligands, much remains unknown about the specificity of ligand binding and how this influences the immune response. It is without a doubt that insight into the specific ligand structure for such CTLs will further assist in understanding how pathogens can either be recognised by the immune system or how they can thwart the immune response. Moreover, the association between CTLs and endogenous ligands can assist in understanding deleterious cellular process such as tumour growth and also regular cellular 'housekeeping' processes, such as debris clearance. Thus, it is imperative that immunologists and chemists continue to work closely together in order to determine how CTL-ligand interactions influence the many varied aspects of the immunology.

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