



## Review

# Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors



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## ABSTRACT

Among myeloid immune receptors, C-type lectin receptors (CLRs) have a remarkable capacity to sense a variety of self and non-self ligands. The coupling of CLRs to different signal transduction modules is influenced not only by the receptor, but also by the nature, density and architecture of the ligand, which can affect the rate of receptor internalization and trafficking to diverse intracellular compartments. Understanding how the variety of self and non-self ligands triggers differential CLR signalling and function presents a fascinating biological challenge. Non-self ligands usually promote inflammation and immunity, whereas self ligands are frequently involved in communication and tolerance. But pathogens can mimic self-inhibitory signals to escape immune surveillance, and endogenous ligands can contribute to the sensing of pathogens through CLRs. In this review, we survey the complexity and flexibility in functional outcome found in the myeloid CLRs, which is not only based on their differing intracellular motifs, but is also conditioned by the physical nature, affinity and avidity of the ligand.

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**Abbreviations:** BDCA, blood DC antigen; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CLR, C-type lectin receptor; CTL, cytotoxic T lymphocyte; CTLD, C-type lectin domain; DAMP, damage-associated molecular pattern; DC, dendritic cell; cDC, conventional DC; dDC, dermal DC; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin; DCAR, DC activating receptor; DCIR, DC inhibitory receptor; Dectin, DC-associated C-type lectin; dLN, draining lymph node; DNGR-1, dendritic cell NK lectin group receptor 1; i.d., intradermal; ICAM, intercellular adhesion molecule; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; MAPK, mitogen-activated protein kinase; MCL, macrophage C-type lectin; MDL, myeloid DAP-12 associated lectin; Mincle, macrophage-inducible C-type lectin; MR, mannose receptor; MSU, monosodium urate; p.i., post-infection; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; ROS, reactive oxygen species; SHP-1, Src homology 2-containing protein tyrosine phosphatase-1; SIGN-R3, specific intracellular adhesion molecule-3 grabbing non-integrin homolog-related 3; Syk, spleen tyrosine kinase; TNF, tumor necrosis factor.

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## Introduction: the C-type lectin domain is a flexible module for endogenous and exogenous ligands

The C-type lectin-like domain (CTLD) (Drickamer, 1999) is a structural motif composed of two loops with conserved cysteines that stabilize the structure with intrachain disulphide bridges. The CTLD was initially defined functionally by its ability to bind carbohydrates in a calcium-dependent manner; however, the conserved structural module is also capable of calcium-dependent binding of glycans, lipids and proteins (Zelensky and Gready, 2005). This review focuses on integral membrane CLR receptors expressed on myeloid cells that modulate functional outcomes (Sancho and Reis e Sousa, 2012) (Table 1).

CLRs are frequently promiscuous receptors that can bind more than one ligand. Myeloid CLRs can act as receptors for endogenous (self) or exogenous (non-self) ligands. Endogenous ligands can be further subdivided into self ligands involved in cell adhesion and communication and those regulating immune homeostasis (García-Vallejo and van Kooyk, 2009) or alternatively can be defined as “altered-self” neoglycans expressed by transformed cells (van Vliet et al., 2008) or “damaged self” danger signals released or exposed upon cell death (Sancho and Reis e Sousa, 2013).

The CLR DC-SIGN (CD209) illustrates the diversity of self and non-self ligands. DC-SIGN binds high mannose and fucose-based ligands (LeX, LeY, LeA, LeB) that can be exposed on a variety of self receptors, such as ICAM-2, ICAM-3, CEACAM-1, Mac1 and CEA, or on non-self proteins such as Salp15 in the saliva of *Ixodes*, Arah1 in *Schistosoma* egg antigen, or structures in pathogens including viruses, bacteria, fungi and eukaryotic parasites (Geijtenbeek et al., 2000, 2003; Gringhuis et al., 2009b; Hovius et al., 2008). The CLR Mincle (CLEC4E) can also bind non-self glycolipids in the cell wall of bacteria and fungi, while detecting self-damage by recognizing SAP-130 exposed and released by necrotic cells (Ishikawa et al., 2009; Schoenen et al., 2010; Wells et al., 2008; Yamasaki et al., 2009) (Ishikawa et al., 2013). Other CLRs detect one ligand that can be expressed in a variety of organisms, as described for Dectin-1 (CLEC7A), which detects  $\beta$ -1, 3 glucans in a variety of bacteria and fungi (Brown, 2006). Some CLRs appear to be restricted to endogenous ligands; for example, DNGR-1 (CLEC9A) detects filamentous F-actin exposed upon necrosis (Ahrens et al., 2012; Sancho et al., 2009; Zhang et al., 2012) and Clec12a senses uric acid crystals formed during cell death (Neumann et al., 2014).

The spectrum of CLR ligands and the structures bearing them is continually expanding and the general view is that most CLRs are adaptable structures that can bind both endogenous and exogenous ligands, resulting in distinct functional outcomes. The diversity of signalling pathways that can be triggered through CLRs depends initially on the cytoplasmic signalling motif, and we proposed a functional classification of signalling CLRs based on these motifs rather than the structure of the CTLD (Sancho and Reis e Sousa, 2012). However, a single receptor can also signal differentially depending on the nature of the ligand; whether the ligand is of low or high affinity, soluble or particulate, or even its particle size will determine the strength and duration of signalling and the functional response. Ligand recognition and signal transduction through the CLR have a major impact on how self and non-self structures modulate myeloid cell function because CLRs work in concert with other innate receptors to control immunity and homeostasis. In this review we examine how the nature of the CLR motif and the bound ligand influence differential signal transduction.

## Do myeloid CLRs transduce an activating or inhibitory signal?

Signalling myeloid CLRs can potentially trigger activating or inhibitory signals, depending on their cytoplasmic signalling motifs

(Sancho and Reis e Sousa, 2012). Some myeloid CLRs bear a hemITAM structure in their cytoplasmic domain, with a single tyrosine within an YXXL motif that couples to Syk (Rogers et al., 2005). Dectin-1, CLEC-2, DNGR-1 and SIGN-R3 belong to this group (Fuller et al., 2007; Huysamen et al., 2008; Rogers et al., 2005; Sancho et al., 2009; Tanne et al., 2009). Dectin-1 behaves as an activating CLR that promotes inflammation and immunity in response to pathogens such as *Candida*. CLEC-2 behaves as an activating receptor in platelets (Fuller et al., 2007) and myeloid cells (Mourao-Sa et al., 2011). SIGN-R3 couples to Syk and signals as an activating receptor (Tanne et al., 2009).

Despite sharing a common hemITAM motif, not all of these CLRs trigger the same transcriptional programme; for example, they might be non-activating in some instances and depending on the context can even trigger inhibitory signals. In a macrophage cell line exposed to zymosan, a chimeric receptor consisting of the extracellular Dectin-1 and intracellular CLEC-2 structures induced Syk-dependent TNF production but not ROS (Kerrigan et al., 2009). SIGN-R3 can modulate signals by other CLRs (Lefèvre et al., 2013). In another study, the intracellular DNGR-1 structure was unable to mediate production of proinflammatory cytokines via the extracellular Dectin-1 domain by dendritic cells (DCs) (Zelenay et al., 2012). The inability of DNGR-1 to act as a myeloid activating receptor is strongly dependent on the presence of an isoleucine at position-1 relative to the tyrosine of the YXXL motif, and mutation of this isoleucine to glycine in the Dectin-1 – DNGR-1 chimera results in increased cytokine production (Zelenay et al., 2012). The function of the hemITAM is defined by its binding to Syk, but the affinity for this binding can be regulated by the particular environment of the YXXL motif. In this regard, the DEDG motif that precedes the YXXL sequence in both Dectin-1 and CLEC-2 might be associated with enhanced association and signalling via Syk (Fuller et al., 2007). Thus not only the presence of the hemITAM but also the particular amino-acid environment around the tyrosine appear to influence the binding of Syk and the subsequent triggering of activating signals downstream of hemITAM-coupled CLRs.

A second group of myeloid CLRs couples to ITAM-bearing domains. MDL-1 (CLEC5A) couples to DAP-12 or DAP-10, which bear ITAM and YINM motifs, respectively (Bakker et al., 1999; Chen et al., 2008; Inui et al., 2009). BDCA-2, DCAR, Mincle and Dectin-2 couple to the Fc $\gamma$  chain, which bears an ITAM motif that recruits Syk and mediates CARD9-dependent activation of NF- $\kappa$ B (Sancho and Reis e Sousa, 2012). MCL couples to Fc $\gamma$  (Miyake et al., 2013), an association that might be mediated by heterodimerization with Mincle (Lobato-Pascual et al., 2013). Although these receptors are considered to be activating, Syk recruitment to ITAM domains can also result in negative signals and inhibition of heterologous receptors. High avidity ligation of ITAM-coupled Fc $\gamma$ s in macrophages triggers a Syk-mediated induction of signalling inhibitors that abrogates TLR responses (Wang et al., 2010). Syk can also inhibit TLR signalling by inducing tyrosine phosphorylation of MyD88 and TRIF, leading to degradation of these adaptor molecules by the ubiquitin ligase Cbl-b (Han et al., 2010). Moreover, Mincle was recently shown to interfere with signalling via Dectin-1 despite both receptors signalling via Syk (Wevers et al., 2014), as we describe below.

Other myeloid CLRs contain ITIM motifs that recruit phosphatases that mediate negative regulation of kinase-associated heterologous receptors, for example the Syk-coupled CLRs. Co-ligation of these receptors with activating receptors results in modulation of the response, and these CLRs are thus candidate pathogen targets for immune escape. Myeloid CLRs included in this group are hDCIR, mDcir1 (DCIR), mDcir2, Clec12a (MCL, DCAL-2, KLRL1, CLL1), MAgH, and Ly49Q (Sancho and Reis e Sousa, 2012). Finally, members of another class of myeloid CLRs do not bear evident ITAM or ITIM domains; these CLRs include MR,

**Table 1**  
Mouse and human myeloid CLR surveyed in this review.

Common name(s)	Gene name	Signalling motifs	Early signalling effectors	Ligand specificity	Ligand origin
DC-SIGN, CLEC4L (Hs)	CD209 (Hs)	YxxL LL EEE	ManLAM: LSP1, KSR1, CNK RAF-1 Salp15: MEK-RAF-1 Fucose: LSP1	High mannose and fucose (Le <sup>X</sup> , Le <sup>Y</sup> , Le <sup>A</sup> , Le <sup>B</sup> )	- HIV-1, measles, Dengue, SARS, CMV, filoviruses - <i>Mycobacterium</i> spp., <i>Lactobacilli</i> spp., <i>H. pylori</i> , <i>E. coli</i> - <i>C. albicans</i> - <i>Leishmania</i> spp. - <i>Ixodes</i> saliva Salp15, <i>Schistosoma</i> egg antigen, Arah1 - ICAM-2, ICAM-3, CEACAM-1, Mac1, CEA - <i>M. tuberculosis</i>
SIGNR3 (Mm)	Cd209d (Mm)	Yxxl	HemITAM-SYK	High mannose and fucose gp120	HIV-1
BDCA-2, DLEC, CD303, CLECSF7 (Hs)	CLEC4C (Hs)	Tmb K EEE	FcRγ chain- ITAM-SYK	ND	ND
DCAR (Mm)	Clec4b1 (Mm)	Tmb R	FcRγ chain- ITAM-SYK	ND	ND
mDCAR1 (Mm)	Clec4b2 (Mm)	Tmb R	FcRγ chain- ITAM-SYK?	ND	ND
DCIR, CLECS-F6, LLIR (Hs) DCIR, Dcir1, Clec6 (Mm)	CLEC4A (Hs) Clec4a2 (Mm)	IxYxxV IxYxxV	SHP-1, SHP-2 SHP-1, SHP-2	ND Syalilated glycoproteins ND	HIV-1 - Syalilated intravenous immunoglobulins ND
Dcir2 (33D1) (Mm) Dectin-2	Clec4a4 (Mm) CLEC6A (Hs) Clec4n (Mm)	IxYxxV Tmb R	ND FcRγ chain- ITAM-SYK Src kinases CARD9	High mannose α-mannans	- <i>M. tuberculosis</i> - <i>A. fumigatus</i> , <i>C. albicans</i> , <i>S. cerevisiae</i> , <i>P. brasiliensis</i> , <i>H. capsulatum</i> , <i>M.</i> <i>audouinii</i> , <i>T. rubrum</i> , <i>C. neoformans</i> - House dust mite allergens, <i>S. mansoni</i> eggs extracts - Ligand on CD4 <sup>+</sup> CD25 <sup>+</sup> T cells - <i>M. tuberculosis</i>
MCL, CLECS-F8	CLEC4D (Hs) Clec4d (Mm)	ND	FcRγ chain through Mincle heterodimer	Glycolipids	- <i>M. tuberculosis</i>
Mincle	CLEC4E (Hs) Clec4e (Mm)	Tmb R	FcRγ chain- ITAM-SYK Src kinases CARD9	Glycolipids, SAP-130.	- <i>C. albicans</i> , <i>Malassezia</i> spp, <i>Fonsecaea</i> <i>pedrosoi</i> . - Dead cells
MDL-1, CLECSF5	CLEC5A (Hs) Clec5a (Mm)	Tmb K	DAP10 – PI3K DAP12-ITAM- SYK	ND	- Dengue virus - Role in osteoclastogenesis: endogenous ligand? - Dead cells.
CLEC12A, M1CL, DCAL-2, KLRL1, CLL1	CLEC12A (Hs) Clec12a (Mm)	VxYxxL	SHP-1, SHP-2	Monosodium urate crystals	- Lymphatic endothelial cells, lymph node stroma, tumor cells, HIV-1 - Snake venom
CLEC-2	CLEC1B (Hs) Clec1b (Mm)	YxxL DEDG LL	HemITAM- SYK; Src and Tec kinases.	- Podoplanin - Rhodocytin	- Dead cells
DNGR-1, CLEC9A	CLEC9A (Hs) Clec9a (Mm)	YxxL	HemITAM-SYK	F-actin	- Dead cells
Dectin-1, β-GR, CLECSF12	CLEC7A (Hs) Clec7a (Mm)	YxxL DEDG	HemITAM-SYK CARD9 RAF-1	β-1,3 glucans	- <i>Mycobacteria</i> spp. - <i>P. carinii</i> , <i>C. albicans</i> , <i>A. fumigatus</i> , <i>Penicillium marneffeii</i> , <i>Coccidioides</i> <i>posadasii</i> , and <i>Histoplasma capsulatum</i> - Ligand on T cells
MR, MMR, CD206	MRC1 (Hs) Mrc1 (Mm)	FxxxxY LL	CDC42, RHOB, PAKs, ROCK1	High mannose, fucose, sLe <sup>X</sup> , GlcNAc	- HIV-1, Dengue - <i>M. tuberculosis</i> , <i>M. kansasii</i> , <i>F.</i> <i>tularensis</i> , <i>K. pneumoniae</i> , <i>S.</i> <i>pneumoniae</i> - <i>P. carinii</i> , <i>C. albicans</i> , <i>C. neoformans</i> - <i>Leishmania</i> spp. - Glycosylated allergens - Lysosomal hydrolases, thyroglobulin, L-selectin, MUC-1, apoptotic cells

DEC-205, DC-SIGN, SIGNR1, Langerin, MGL, CLEC-1, DCAL-1, LOX-1 and LSECtin. DC-SIGN is the archetypal receptor of this group and its triggering results in modulation of signalling by heterologous receptors (Sancho and Reis e Sousa, 2012).

An additional source of complexity in signal transduction through myeloid CLR is their capacity to form dimers or multimers. Modelling of CLEC-2 signalling through hemITAMs indicates that Syk binding requires phosphorylation of paired YXXL motif tyrosines in a homodimer. Stoichiometric analyses show that

CLEC-2 pre-exists as a dimer which then aggregates after ligand binding (Hughes et al., 2010; Watson et al., 2009). Some CLR can also heterodimerize; for example, MCL forms a functional heteromeric complex with Mincle and FcRγ (Lobato-Pascual et al., 2013). Through this interaction, MCL acquires signalling capacity via FcRγ, whereas Mincle benefits from the endocytic capacity of MCL. Formation of the heterodimer could also potentially increase ligand binding affinity or expand ligand specificity (Yamasaki, 2013).

## The nature of the ligand can modulate signals through myeloid CLR

In addition to the signalling motifs borne by the CLR, signalling can also be influenced by the nature of the ligand, which can alter receptor engagement or induce conformational changes. CLR frequently bind more than one ligand, each with a distinct affinity and avidity for the receptor. The importance of the nature of the ligand is well illustrated by signalling through DC-SIGN. Following binding of mannosylated lipoarabinomannan from *Mycobacterium tuberculosis*, DC-SIGN couples to a LSP1 – KSR1 – CNK signalosome, leading to activation of Raf-1 and acetylation of the NF- $\kappa$ B p65 subunit, which results in enhanced proinflammatory responses (Gringhuis et al., 2009a). High mannose ligands from *Candida* or HIV-1 behave similarly and induce Raf-1 activation, whereas fucose-based ligands cause dissociation of the LSP1-based signalosome, so that DC-SIGN associates only with LSP1 and proinflammatory responses are suppressed (Gringhuis et al., 2009a). Salp15, a protein produced by the salivary glands of *Ixodes scapularis* ticks, also binds DC-SIGN to promote Raf-1 activation, in this case leading to MEK activation and suppression of inflammation (Hovius et al., 2008).

Ligand affinity and avidity can affect the quantity and duration of signals through the ITAM domain, resulting in differential responses (Hamerman et al., 2009; Yamasaki et al., 2004) (Fig. 1A). The Fc $\gamma$ R chain contains a consensus ITAM with two tyrosines that upon binding of a high avidity ligand becomes fully phosphorylated and associates with the kinase Syk triggering an activating signal that contributes to immunity and inflammation (Mócsai et al., 2010). However, when ITAM-coupled receptors associate with low affinity or avidity ligands, the ITAM domain is hypophosphorylated and this results in preferential association with SH2-containing phosphatases, a configuration termed inhibitory ITAM (Blank et al., 2009). The paradigm of the differential effect of low affinity and avidity is the Fc $\alpha$ RI receptor, which associates with the Fc $\gamma$ R chain for signalling. Upon interaction with IgA monomers, the coupled Fc $\gamma$ R chain is partially phosphorylated and recruits SHP-1 phosphatase, being able to inhibit signals through heterologous receptors (Pasquier et al., 2005). Some CLR that couple to the Fc $\gamma$ R chain, including Mincle, Dectin-2, BDCA-2, DCAR or MCL, could thus potentially act in a similar manner to the Fc $\alpha$ RI receptor and trigger phosphatase signalling upon binding to a low affinity/avidity ligand, which would lead to inhibition of signals triggered through heterologous receptors that could themselves be CLR signalling via Syk. In this way, ITAM-associated CLR could respond to low valency ligation by soluble extracellular ligands that would induce broad suppression and dampening of inflammation and immunity. This recognition of low affinity/avidity ligands by ITAM-coupled receptors would thus act similarly to ITIM-coupled CLR, which trigger a negative signal upon co-ligation with heterologous receptors by multivalent ligands, selectively regulating the co-ligated receptor (Hamerman et al., 2009).

Affinity is also influenced by whether the ligand exists in a monomeric or oligomeric state, which affects how the ligand is presented to the CLR (Fig. 1B). Soluble ligands for CLR are poor triggers of activating signalling, whereas the same ligands in plated form promote Syk signalling effectively. This might provide a mechanism to restrict activation of the tightly regulated processes of phagocytosis and ROS production to situations when the host is in direct contact with pathogen. In this regard, Dectin-1 has been proposed to cluster in a “phagocytic synapse” only when it interacts directly with particulate, but not soluble,  $\beta$ -glucans (Goodridge et al., 2011). CD45 and CD148 phosphatases are initially needed for dephosphorylation of the Src family kinase Lyn at its inhibitory tyrosine (Y507), but are subsequently excluded from the  $\beta$ -glucan particle contact site to permit Syk association and signalling (Goodridge et al., 2011). This formation of a phagocytic synapse that excludes

membrane phosphatases may be a general feature of other phagocytic CLR, which would thus discriminate between detection of soluble and particulate ligands (Fig. 1C). After detection of soluble ligands, the inhibitory activity of membrane tyrosine phosphatases would not be segregated from the receptors, and CLR signalling would be attenuated. As mentioned above, non-receptor phosphatases such as SHP-1 could also dampen CLR signalling and, depending on ligand affinity, compete with Syk for binding to the phosphorylated tyrosine.

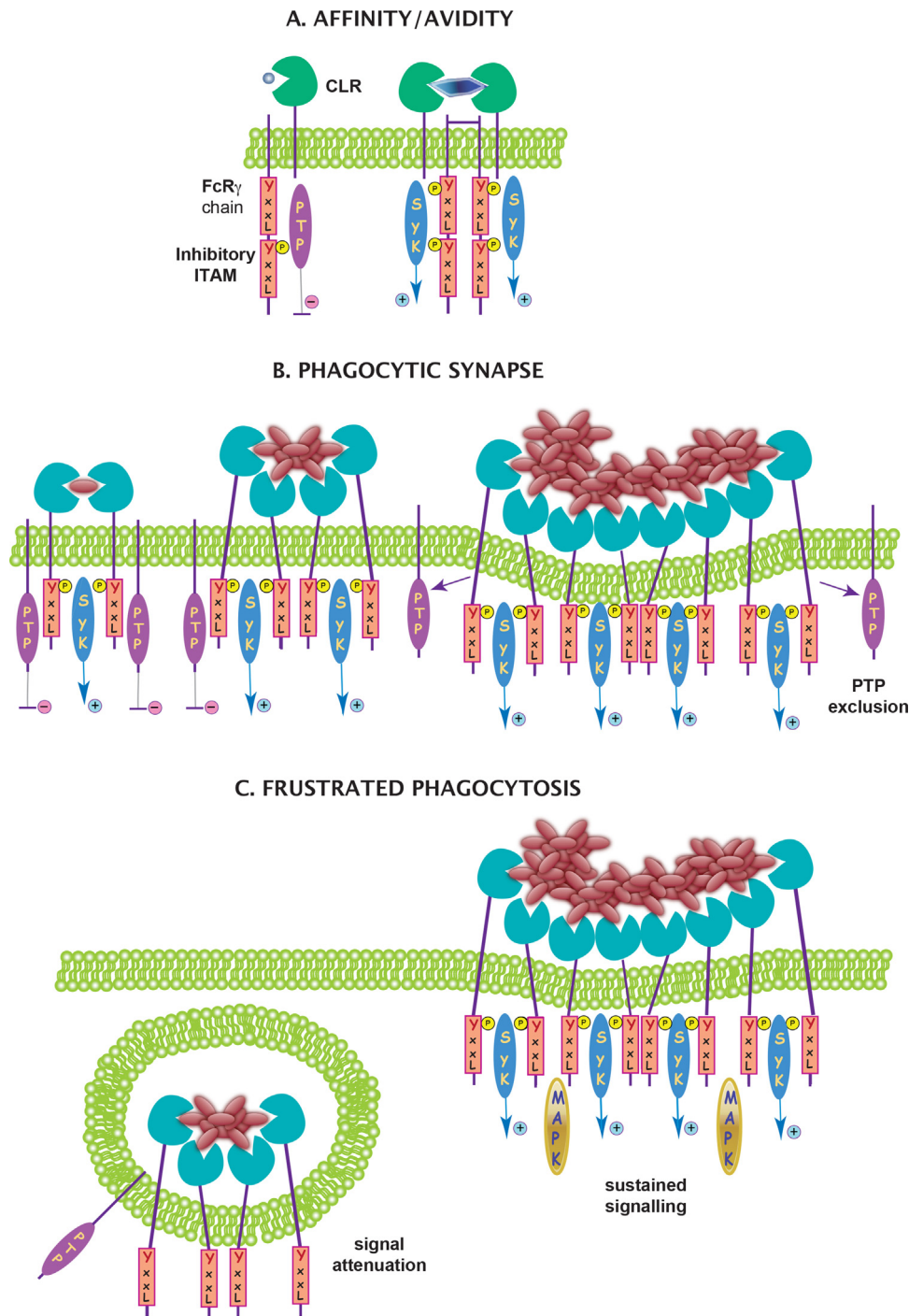
CLR signalling is also influenced by the size of the particle on which the ligand is expressed. Presentation of CLR ligands on large particles rather than in solution or even on small particles could promote delayed or “frustrated” phagocytosis, which results in an enhanced cytokine response (Hernanz-Falcón et al., 2009; Rosas et al., 2008) (Fig. 1C). Large aggregates of microparticulate  $\beta$ -glucan, known as Curdlan particles, are much more potent than non-aggregated  $\beta$ -glucan microparticulates at inducing signalling and inflammatory responses via Dectin-1 expressed on DCs or macrophages (Rosas et al., 2008). The poor ability of  $\beta$ -glucan microparticles to trigger potent IL-6 or TNF production via Dectin-1 can be reverted by the actin polymerization inhibitor cytochalasin D, which blocks phagocytosis of the microparticles and thus promotes inflammatory signals (Rosas et al., 2008). Blockade of ligand-driven internalization of Dectin-1, either with large  $\beta$ -glucan particles or with inhibitors of actin polymerization or dynamin, results in sustained MAPK activation (Hernanz-Falcón et al., 2009), suggesting that endocytosis of the receptor leads to attenuation of inflammatory responses. This attenuation could be mediated by disassembly of signalling complexes upon receptor internalization or by improved access of inhibitory factors (e.g. phosphatases) to the signalling complex. Other Syk-dependent signals, however, such as ROS production, appear to be regulated differently since they are active upon receptor internalization (Underhill et al., 2005). Plated ligands probably mimic this “frustrated” phagocytosis, resulting in exaggerated signalling.

## Sensing of self and non-self by myeloid CLR: plasticity of functional outcomes

We describe above how the complexity of signalling motifs and ligand interaction with myeloid CLR results in distinct functional outcomes. In this section, we illustrate how self and non-self ligands target CLR. We present examples of how myeloid CLR detect self for homeostasis or promotion of tolerance, whereas detection of damaged-self contributes to immunity. When detecting non-self, myeloid CLR also act as innate receptors that trigger immunity and inflammation. However, in certain circumstances pathogens exploit the versatility of CLR for immune evasion.

### Sensing non-self to favour immunity and inflammation

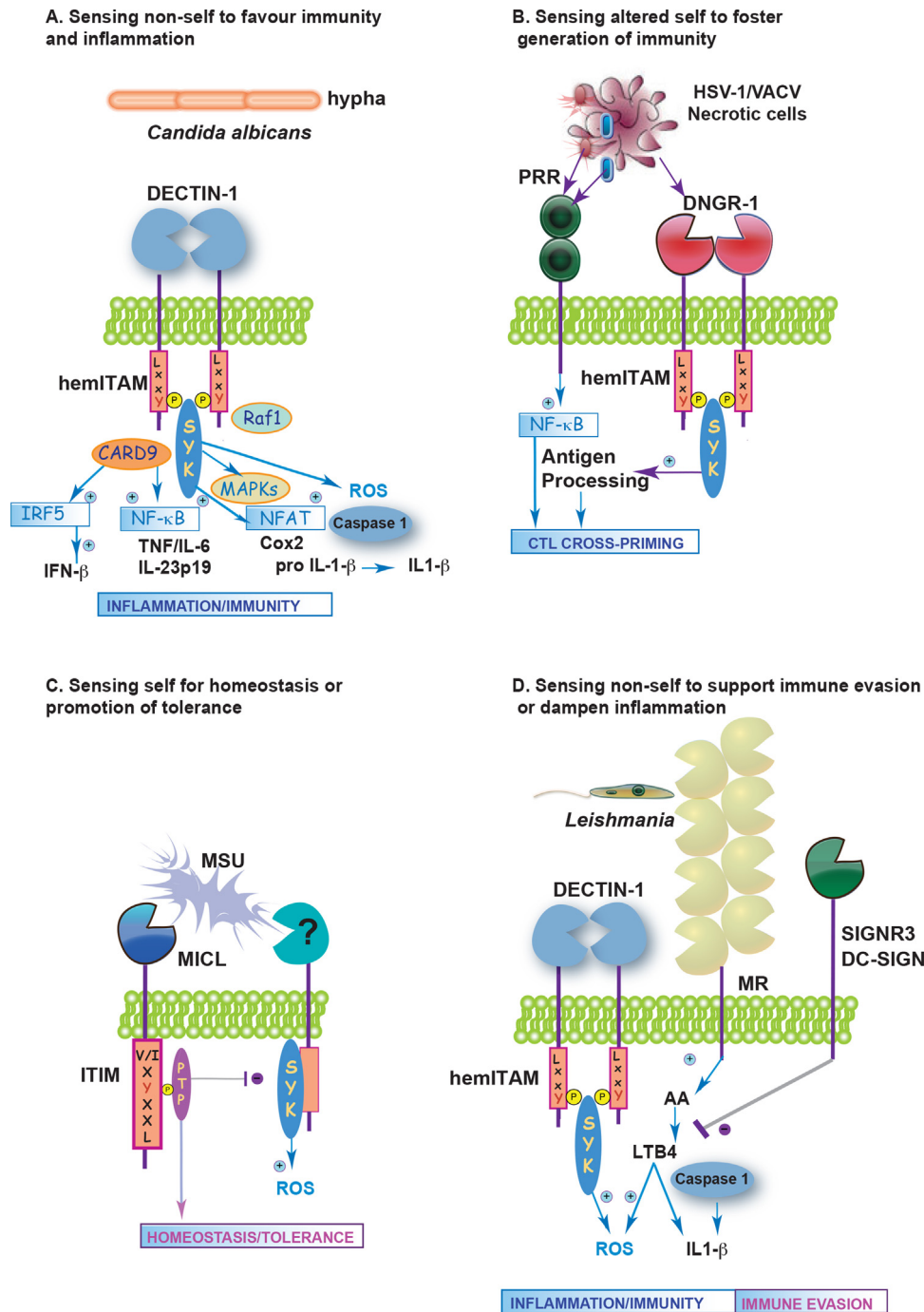
The immune system has evolved to protect the host from infection. Charles Janeway hypothesized the existence of pattern recognition receptors (PRRs) as sensors of pathogen-associated molecular patterns (PAMPs) and inducers of the co-stimulatory capacity of DCs (Janeway, 1989). The induction of a signalling pathway by these PRRs leads to innate cell activation, which in turn orchestrates an appropriate inflammatory response and instructs the nature of the adaptive response. Most pathogen-detecting CLR are phagocytic or scavenger-type receptors, and as such contribute to an immune response but are not primary drivers. The archetypal example of a myeloid CLR able to initiate immunity is Dectin-1, which recognizes  $\beta$ -glucans present in the cell wall of many fungi (Brown, 2006) (Fig. 2A). The phosphorylated tyrosine in the hemITAM recruits Syk kinase, which in turn activates the CARD9/Bcl10/Malt-1 module



**Fig. 1.** The nature of the ligand can modulate signalling through myeloid CLR. Signalling myeloid CLR can potentially trigger activating or inhibitory signals depending on their cytoplasmic signalling motifs and the nature of their ligands. (A) Affinity and avidity of the ligand can affect the quantity and duration of signals through the ITAM domain. Low affinity/avidity ligands induce hypophosphorylation of the ITAM domain in the FcR $\gamma$  chain associated with the CLR. The hypophosphorylated ITAM, termed “inhibitory ITAM”, preferentially binds SH2-containing protein tyrosine phosphatases (PTPs). Upon binding of a high avidity ligand, the FcR $\gamma$  chain ITAM becomes fully phosphorylated and associates with Syk kinase, which triggers an activating signal. (B) Soluble and particulate ligands are differentially sensed by CLR. Soluble ligands for CLR are poor triggers of activating signalling because of the inhibitory activity of membrane PTPs. When a phagocytic synapse is formed following binding of particulate ligands, these phosphatases are segregated from the receptors. (C) CLR signalling is influenced by the size of the ligand-bearing particle. Small particles are endocytosed, resulting in attenuation of signalling. Delayed or “frustrated” phagocytosis of large particles enhances signalling and inflammatory responses.

that activates the I $\kappa$ B kinase (IKK) complex to induce canonical NF- $\kappa$ B signalling (Gross et al., 2006). Syk can also activate MAPK (Leibundgut-Landmann et al., 2007; Slack et al., 2007) and NFAT (Goodridge et al., 2007), which collaborate with NF- $\kappa$ B in the activation of a transcriptional programme. Human Dectin-1 also induces a Syk-independent pathway mediated by the serine/threonine

protein kinase Raf-1, which results in acetylation of NF- $\kappa$ B and subsequent modulation of transcription (Gringhuis et al., 2009b). Activation of these signalling pathways induces transcription of genes encoding proinflammatory cytokines such as TNF, IL-6 and IL-23 and the regulatory cytokines IL-2 and IL-10 (Leibundgut-Landmann et al., 2007), a pattern somewhat different from the



**Fig. 2.** Self and non-self sensing by myeloid CLR: plasticity of functional outcomes. The complexity of signalling motifs and ligand interaction with myeloid CLR can result in distinct functional outcomes: (A) Sensing non-self to favour immunity and inflammation: Dectin-1 detects  $\beta$ -glucan carbohydrates on *Candida albicans*. After ligand binding, Dectin-1 can mediate multiple cellular responses in a Syk-dependent or independent (Raf1) manner, including cytokine and chemokine production, respiratory burst, phagocytosis and IFN- $\beta$  production. (B) Sensing altered self to foster generation of immunity: Cells infected with cytopathic viruses (HSV-1 or VACV) can be detected by TLRs and DNGR-1 expressed on DCs. Whereas PRRs promote activation of the DCs, DNGR-1 favours CTL priming by sequestering cargo in a poorly degradative early endocytic compartment that allows MHC class I cross-presentation. (C) Sensing self for homeostasis or promotion of tolerance; Monosodium urate (MSU) is a damage-associated molecular pattern (DAMP) that induces Syk-dependent ROS production. Clec12a can sense MSU and promote an inhibitory signal through its ITIM that dampens inflammation. (D) Sensing non-self to support immune evasion or dampen inflammation: During *Leishmania infantum* infection, Dectin-1 and MR induce ROS production in macrophages and trigger Syk-coupled secretion of IL-1 $\beta$ . SIGNR3 (DC-SIGN in human macrophages) promotes parasite survival by inhibiting the LTB4/IL-1 $\beta$  axis.

cytokines stimulated by TLRs. The specific Dectin-1 agonist Curdlan acts as an adjuvant to trigger an adaptive response *in vitro* and *in vivo*, leading to Th1 and Th17 type immunity (Leibundgut-Landmann et al., 2007). In addition, sensing of *Candida albicans* by Dectin-1 and Dectin-2 results in activation of IFN- $\beta$  production through a Syk-IRF5 axis (del Fresno et al., 2013). Dectin-2 is

the predominant Syk-coupled receptor in the response of DCs to *C. albicans*, and induction of Th17 immunity against the fungus in mouse models (Robinson et al., 2009; Saijo et al., 2010). Apart from inducing transcriptional responses, both Dectin-1 and Dectin-2 mediate Syk-dependent ROS generation (Gross et al., 2006; Ritter et al., 2010; Underhill et al., 2005) which has a direct microbicidal

role and triggers the NLRP3 inflammasome, leading to caspase-1 activation and processing of pro-IL-1 $\beta$  to IL-1 $\beta$  essential for anti-fungal immunity (Gross et al., 2009).

Sensing non-self to induce immunity and inflammation confers the host with an advantage in the fight against a pathogen, but is an undesired outcome when the non-self triggering agent is an allergen. Allergenic extracts from *Aspergillus fumigatus* or house-dust mites bind Dectin-2 and trigger Syk-dependent arachidonic acid metabolism and rapid production of cysteinyl leukotrienes (Barrett et al., 2009). These lipid mediators promote Th2 responses with increased eosinophilia and neutrophilia in the lung (Barrett et al., 2011). Another setting in which sensing non-self to promote inflammation can be deleterious to the host is during viral infections with severe immunopathology, for example sensing of Dengue virus by MDL-1 (CLEC5A), which results in phosphorylation of DAP12 ITAM and generation of a Syk-dependent proinflammatory response (Chen et al., 2008). Exaggerated inflammation results in a shock syndrome, with plasma leakage and subcutaneous and vital organ haemorrhaging. Blockade of the MDL-1–Dengue virus interaction in a mouse model suppresses the secretion of pro-inflammatory cytokines by macrophages, preventing the excessive immunopathology (Chen et al., 2008).

#### *Sensing self to foster generation of immunity*

In addition to directly sensing pathogens, the immune system also responds to danger, as demonstrated by adaptive immune responses to damaged tissue or necrotic cells in apparently infection-free situations. The model of innate recognition of dangerous-self proposes that preformed endogenous adjuvants inside healthy cells, known as damage-associated molecular patterns (DAMPs), are exposed or released upon necrotic cell death (Matzinger, 1994). The sensing of damaged cells by DCs has attracted much interest in understanding how these pathways might be exploited to improve vaccine design. The CLR DNGR-1 (CLEC9A) is selectively expressed on DC subsets specialized in cross-presentation of antigens from dead cells in mice and humans (Caminschi et al., 2008; Huysamen et al., 2008; Poulin et al., 2010; Sancho et al., 2008). DNGR-1 recognizes F-actin complexed to the actin-binding domain of certain cytoskeletal molecules (Ahrens et al., 2012; Zhang et al., 2012). To date, no other ligands for DNGR-1 have been found in pathogens, although it has been found to bind fungal F-actin (Ahrens et al., 2012). It could be argued that DNGR-1 is non-essential for immunity to pathogens. However, our work demonstrated that cells infected with vaccinia virus (VACV) eventually expose DNGR-1 ligands (Iborra et al., 2012) (Fig. 2B); while dispensable for activation of DCs, DNGR-1 was crucial for cross-presentation of necrotic cargo associated with VACV or herpes simplex virus-type 1 (HSV-1) infected cells (Iborra et al., 2012; Zelenay et al., 2012). DNGR-1 thus plays a non-redundant role in the cross-priming of CTLs in models of cytopathic infection with HSV-1 or VACV (Iborra et al., 2012; Zelenay et al., 2012). These results suggest that DNGR-1 diverts necrotic cargo away from lysosomes, targeting them for accumulation in a pre-lysosomal compartment devoted to cross-presentation (Iborra et al., 2012; Zelenay et al., 2012). Sensing of damaged self during viral infection by DNGR-1 favours antigen processing for cross-presentation and cooperates with PRRs that mediate activation of DCs to promote CTL immunity. Moreover, poxviruses and other intracellular pathogens are propelled beneath the plasma membrane into surrounding cells by growing actin tails (Welch and Way, 2013) that are brightly stained with the DNGR-1 extracellular domain (Ahrens et al., 2012). Since these actin tails containing DNGR-1 ligand are exposed on damaged infected cells, one could hypothesize that DNGR-1 has evolved during host–intracellular pathogen co-evolution to detect this danger signal on infected cells and favour host responses. This

interaction would allow non-infected healthy DCs to take up and process antigens that can then cross-prime CTL responses.

#### *Sensing self for homeostasis or promotion of tolerance*

In addition to the inflammation and immunity that can result from sensing of dangerous-self, self-sensing could also potentially lead to tolerance or prevention of immunopathology. It has been proposed the existence of “self-associated molecular patterns” (SAMPs), which would be recognized by intrinsic cognate inhibitory receptors self-PRRs (SPRRs) in order to maintain the homeostatic non-activated state of innate immune cells and dampen their reactivity (Elward and Gasque, 2003; Varki, 2011). An example of these putative SAMPs is provided by sialic acid patterns, which are detected by SPRRs such as Siglecs (sialic acid recognizing Ig-like lectins). These SPRRs often have ITIM motifs within their cytosolic tails. Consistent with this idea, deletion of Siglec-F from mouse eosinophils produces a hyperactive response (Zhang et al., 2007), and mouse Siglec-G deletion results in over-reactive responses to DAMPs and PAMPs (Chen et al., 2009).

One putative SPRR among CLRs is DCIR. This receptor is expressed mainly on DCs and has an extracellular carbohydrate recognition domain and an ITIM motif. Autoimmune-related genes map to the DCIR locus in humans, and aged DCIR-deficient mice spontaneously develop sialadenitis and enthesitis associated with elevated serum autoantibodies. These mice also showed a markedly exacerbated response to collagen-induced arthritis. Thus, DCIR behaves as a SPRR, recognizing a self-associated pattern and maintaining immune system homeostasis (Fujikado et al., 2008). Notably, DCIR can be targeted by intravenous immunoglobulins expressing sialic acid and mediates a negative signal in DCs via SHP-2 and SHIP-1, resulting in the generation of Tregs and attenuation of allergic airway disease (Massoud et al., 2014).

Clec12a was recently identified as another CLR that mediates an anti-inflammatory response through recognizing a well-established DAMP, uric acid (Neumann et al., 2014) (Fig. 2C). Uric acid is soluble in healthy cells, but forms monosodium urate (MSU) crystals when it comes into contact with extracellular sodium ions upon cell death. MSU activates Syk signalling in myeloid cells through direct interaction with the membrane or through unidentified mechanisms involving CD16 and CD11b (Barabe et al., 1998; Desaulniers et al., 2001; Ng et al., 2008). Human and mouse Clec12a both sense MSU (Neumann et al., 2014), although it is not fully understood how this recognition works. MSU induces Syk-dependent production of reactive oxygen species (ROS), and Clec12a inhibits this production in neutrophils (Neumann et al., 2014). Injection of MSU crystals into the peritoneum of mice induces neutrophil infiltration that is augmented in Clec12a-deficient mice. Notably, Clec12a does not affect ROS production induced by Dectin-1, suggesting that, to induce its inhibitory effects, Clec12a requires co-engagement with the activating receptor for MSU. Because Clec12a includes an ITIM within its cytoplasmic tail, its negative regulatory role is likely to be mediated by recruited phosphatases.

#### *Sensing non-self to support immune evasion or dampen inflammation*

The proposal that SAMPs dampen inflammation in response to self antigens opens up a possible route for pathogens to mimic SAMPs for their own benefit. For example, the *Trypanosoma cruzi* cell surface contains sialoglycoproteins and sialyl-binding proteins that assist the parasite in several functions including parasite survival, infectivity, and host–cell recognition, and could act as SAMPs (Freire-de-Lima et al., 2012). ITIM-coupled CLRs such as DCIR could behave as SPRRs that are targeted directly or

indirectly (e.g. by tissue damage) by pathogens to dampen immunity and inflammation. In this regard, DCIR-deficient mice (*Clec4a2*<sup>-/-</sup>) exhibit severe inflammatory disease upon Chikungunya virus infection (Long et al., 2013). Early differences in virus-induced disease between DCIR-deficient and wild-type mice are independent of viral replication, and there is no evidence for direct interactions between DCIR and Chikungunya virus, suggesting that DCIR might sense the pathogen indirectly and contribute to increased tolerance of pathogen-induced damage by dampening immunopathology and reducing the negative impact of the pathogen on host fitness (Medzhitov et al., 2012). Conversely, in a model of cerebral malaria caused by *Plasmodium berghei*, DCIR-deficient mice are more resistant, correlating with reduced T-cell priming in the spleen, decreased TNF levels in the serum, and diminished T-cell retention in the brain (Maglinao et al., 2013). No evidence was found for direct binding of DCIR to *P. berghei*, suggesting that DCIR recognizes a self-DAMP released by the rupture of red blood cells during infection. These studies highlight the potential plasticity of DCIR depending on the ligand and the inflammatory context.

As discussed above, inhibitory signals can also be mediated by ITAM-coupled receptors. The ITAM-coupled CLR Mincle, in addition to sensing SAP-130 – a self-ligand released by necrotic cells (Yamasaki et al., 2008) – binds glycolipids in the cell walls of fungi and bacteria (Ishikawa et al., 2009, 2013; Schoenen et al., 2010; Sousa et al., 2011; Yamasaki et al., 2009). Mincle is an activating receptor that upon ligand recognition triggers a strong Syk-dependent inflammatory response and robust Th1 and Th17 immunity (Ishikawa et al., 2009, 2013; Schoenen et al., 2010; Shenderov et al., 2013; Sousa et al., 2011; Wells et al., 2008; Yamasaki et al., 2009). However, in certain conditions Mincle can deliver a negative signal to heterologous receptors. Mincle and Dectin-1 recognize *Fonsecaea monophora*, a cause of chromoblastomycosis. Stimulation of human DCs with *F. monophora* triggers the maturation of DCs and production of IL-6, IL-1 $\beta$  and IL-23, but not IL-12p70. While Dectin-1 acts as a PRR, promoting Syk-dependent cytokine production, stimulation of Syk by Mincle triggers a PKB-dependent signal that leads to loss of IRF1 activity (Wevers et al., 2014). Notably, Mincle suppresses TLR-4-induced IL-12p70 expression by inhibiting IL-12p35 transcription (Wevers et al., 2014). These findings suggest that Mincle can support or inhibit immunity depending on the nature of the ligand, thus modulating the response of heterologous receptors for the same pathogen. Mincle is also involved in innate sensing of *F. pedrosoi* in mice, but this recognition does not seem to be efficient enough to promote protective immunity against the fungus, instead leading to chronic infection (Sousa et al., 2011). Intriguingly, when mice are treated with TLR agonists, TLR signalling cooperates with Mincle in the generation of a potent inflammatory response that resolves the disease (Sousa et al., 2011). The reasons for this apparent discrepancy between humans and mice are unknown, but it could imply the existence of additional receptors that heterodimerize with Mincle in mouse, as described for MCL (Miyake et al., 2013), or of different transduction modules associated with human monocyte-derived DCs compared with mouse bone marrow-derived DCs. In this regard, the ability of a given CLR to drive NF- $\kappa$ B activation is myeloid-cell-type dependent. DCs derived from bone marrow progenitors and differentiated *in vitro* with GM-CSF are easily activated by Dectin-1 agonists, whereas Flt3L-derived bone marrow DCs and M-CSF-derived macrophages are poor NF- $\kappa$ B activators (Goodridge et al., 2009). This difference might be explained by the expression levels of CARD9 or other key limiting signalling proteins. Thus, depending on the cellular context, the simultaneous engagement of CLRs and TLRs could lead to cooperation or antagonism.

DC-SIGN recognizes endogenous glycoproteins and also interacts with a wide range of pathogens through the recognition of

mannose and fucose, but its contribution to host defence is still debated. DC-SIGN can contribute to adaptive immunity by targeting antigens to late endosomal/lysosomal compartments for degradation and presentation to T cells. But DC-SIGN also induces signals that promote HIV-1 replication in DCs and transmission to T cells (Hodges et al., 2007; Kwon et al., 2002). Similarly, the interaction of *M. tuberculosis* with DC-SIGN suggests that the pathogen has evolved to exploit the receptor as part of an immunoevasive strategy involving the secretion of the immunosuppressive cytokine IL-10 from DCs (Geijtenbeek et al., 2003). Mannose-containing *M. tuberculosis* and HIV-1 induce the recruitment of the LSP1 signalosome described above, which activates Raf-1 and modulates TLR signalling (Gringhuis et al., 2007, 2009a). The DCs of transgenic mice expressing human DC-SIGN under the control of the mouse CD11c promoter (termed hSIGN) produce significantly less IL-12p40 than wild-type counterparts and similar amounts of IL-10. After infection with *M. tuberculosis*, hSIGN mice show massive accumulation of DC-SIGN<sup>+</sup> cells in infected lungs, below-normal tissue damage and prolonged survival. These results suggest that, rather than promoting immune evasion by mycobacteria, human DC-SIGN may have evolved to limit tuberculosis-induced pathology (Schaefer et al., 2008). Similarly, the DC-SIGN mouse homolog SIGNR3 dampens inflammation mediated by other CLRs that sense *Leishmania* infection (Lefèvre et al., 2013) (Fig. 2D). Macrophages act as the main host and primary effector cells during *Leishmania* infection. The macrophage response to *Leishmania infantum* *in vivo* is characterized by M2b polarization (Mantovani et al., 2004) and is detected by Dectin-1, mannose receptor (MR), and SIGNR3. Dectin-1 and MR respectively activate Syk-p47phox and arachidonic acid-NADPH oxidase signalling pathways, both of which are needed for ROS production, and also trigger Syk-coupled signalling for caspase-1-induced IL-1 $\beta$  secretion. Dectin-1 and MR thus contribute to parasite containment, whereas SIGNR3 favours parasite resilience through inhibition of the LTB4-IL-1 $\beta$  axis.

## Concluding remarks

Myeloid CLRs detect self and non-self to mediate multiple roles. The outcome of myeloid CLR interaction with a ligand depends critically on the signals triggered, which are conditioned by the signalling motifs. The classical view of activating ITAMs and inhibitory ITIMs is complicated by the ability of low ligand density to lead to inhibitory ITAM signalling. Additionally, under certain circumstances Syk-dependent responses can also lead to inhibition of gene transcription. Soluble ligands also result in weaker signalling than particulate ligands, which form a synapse that excludes inhibitory phosphatases. In addition, large particles that frustrate phagocytosis can signal for longer than small particles, whose signal is attenuated by endocytosis.

This complex and versatile regulation of functional responses depends on the ligand and the signalling motifs present in the receptor and leads to a great variety of responses to self and non-self antigens. Myeloid CLRs mediate immunity and inflammation against non-self, in collaboration with the detection of dangerous-self signals. However, CLRs can also be targeted by pathogens and self ligands to dampen inflammation and immunity. These functions might have been selected in evolution to reduce immunopathology by increasing host tolerance of pathogen-induced damage, providing an alternative strategy to reducing the negative impact of the pathogen on host fitness. Further research is needed to clarify the contribution of ligands and signalling motifs to the functional outcomes of signalling through myeloid CLRs, with the aim of identifying novel targets for improving immunity against pathogen or, conversely, reducing the immunopathology associated with infection.



## Conflict of interest

The authors declare no commercial or financial conflict of interest.

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