Flexible Signaling of Myeloid C-Type Lectin Receptors in Immunity and Inflammation

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Myeloid C-type lectin receptors (CLRs) are important sensors of self and non-self that work in concert with other pattern recognition receptors (PRRs). CLRs have been previously classified based on their signaling motifs as activating or inhibitory receptors. However, specific features of the ligand binding process may result in distinct signaling through a single motif, resulting in the triggering of non-canonical pathways. In addition, CLR ligands are frequently exposed in complex structures that simultaneously bind different CLRs and other PRRs, which lead to integration of heterologous signaling among diverse receptors. Herein, we will review how sensing by myeloid CLRs and crosstalk with heterologous receptors is modulated by many factors affecting their signaling and resulting in differential outcomes for immunity and inflammation. Finding common features among those flexible responses initiated by diverse CLR-ligand partners will help to harness CLR function in immunity and inflammation.

Keywords: lectin receptors, signaling, monocytes, macrophages, dendritic cells, innate immunity, inflammation

DIVERSITY OF SIGNALING MODULES IN MYELOID C-TYPE LECTIN RECEPTORS (CLRs)

The expression of diverse pattern recognition receptors (PRRs), including differential expression of CLRs, provides different subsets of immune cells with a repertoire to interpret and respond distinctly to the information coming from the environment. Myeloid cells are central for initiation and regulation of innate and adaptive immunity or tolerance and the CLR repertoire essentially contributes to myeloid cell function. We previously proposed a classification of myeloid CLRs based on their intracellular signaling motifs (1). While signaling motifs allow to predict effector responses following sensing by CLRs, this canonical response is subjected to modulation by the physical nature, affinity, and avidity of the ligand (2). Based on their intracellular signaling motifs, myeloid CLRs can be classified into the following broad categories (Figure 1): immunoreceptor tyrosine-based activating motif (ITAM)-coupled CLRs, hemi-ITAM-(hemiITAM)-bearing CLRs, immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing CLRs, and a group of CLRs lacking typical signaling motifs (1, 3, 4).

Immunoreceptor tyrosine-based activating motif-coupled CLRs have a classical ITAM motifs in their intracellular tail, consisting of YXXL tandem repeats, or can interact with ITAM-containing adaptor proteins, as Fc receptor γ (FcγR) chain or DNAX-activation protein 12 (DAP12) (5). The majority of them, including Dectin-2 (CLEC6A in human, Clec4n in the mouse), Mincl (CLECAE), MCL (CLECAD), BDCA-2 (human CLEC4C), DCAR (mouse Clec4b1), DCAR1 (mouse Clec4b2), and mannose receptor (MR) (MRCl, CD206) utilize the FcγR chain adaptor, while MDL-1...
Figure 1 | Canonical signaling modules in myeloid C-type lectin receptors (CLRs). Based on canonical intracellular signaling motifs, myeloid CLRs can be classified into immunoreceptor tyrosine-based activating motif (ITAM)-coupled CLRs, hemi-ITAM-(hemITAM)-bearing CLRs, immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing CLRs, and a group of CLRs lacking typical signaling motifs. Mincle, Dectin-1, DCIR, DC-SIGN, and their corresponding canonical signaling pathways and adaptors are depicted as prototypical examples of each category.
example, DC-SIGN intracellular tail is associated with a lysosome composed of the scaffold proteins LSP1, KSR1, and CNK and the kinase Raf-1 in unstimulated DCs (59) (Figure 1). Similar to other CLRs in this group, DC-SIGN cannot promote DCs activation or cytokine secretion per se, but it rather modulates signaling by heterologous receptors (see below) or engages the endocytic machinery contributing to antigen processing and presentation to T cells (3).

Along this review, we will provide illustrative examples of how signaling pathways triggered by a CLR coupled to a particular canonical motif can vary depending on many factors. We will focus on Mincle, Dectin-1, DNGR-1, DCIR, and DC-SIGN as myeloid CLRs representative of each category of signaling motif. Table 1 includes the signaling module coupled to each CLR surveyed in this review, common and gene names, category of flexible signaling source, signaling pathway involved, and the inflammatory outcome provided by such flexibility. In this Table 1, CLRs are grouped based on the signaling module they bear (left column) and graphically illustrates how the signaling pathways triggered by these receptors are more complex and versatile (right columns) than expected by their signaling modules.

### Table 1 | Myeloid C-type lectin receptors (CLRs) surveyed in this review.

| Signaling module | Common name | Gene name | Source of flexible signaling | Signaling pathway | Flexibility outcome |
|------------------|-------------|-----------|-------------------------------|------------------|--------------------|
| No immunoreceptor tyrosine-based activating motif (ITAM) or immunoreceptor tyrosine-based inhibitory motif (ITIM) | CD209 | DC-SIGN | Homotetramerization | LSP1–KSR1–CNK–Raf-1 | Intrinsic (80) |
|                  |             |           | Sensing self and non-self     | LSP1–κκκκ–Bcl3    | Inhibitory (113–115) |
|                  |             |           | Heterologous modulation       | KSR1–CNK–Raf-1    | Activating (69, 110) |
|                  |             |           |                                | (?)               | Activating (148) |
|                  |             |           |                                | Raf-1–MEK         | Inhibitory (112) |
| ITAM             | MDL-1       | CLEC5A    | Heterotrimerization DC-SIGN/MR/MDL-1 | DNA-activation protein 12 (DAP12) | Activating (86) |
|                  | Mannose receptor (MR) | MRC1 | Heterotrimerization DC-SIGN/MR/MDL-1 | DAP12            | Activating (86) |
|                  |             |           |                                | DAP12            | Activating (69) |
|                  | Dectin-2    | CLEC6A, Clec4n | Inhibitory ITAM | Fc receptor γ (FcRγ)–Grb2–SHP-1 | Inhibitory (12) |
|                  |             | CLEC4D    | Heterodimerization Dectin-2/MCL | FcRγ–spleen tyrosine kinase (Syk)–NF-κB p65 | Activating (85) |
|                  | MCL         | CLEC4E    | Heterodimerization Mincle/MCL | FcRγ–Syk         | Activating (81–84) |
|                  | Mincle      | CLEC4E    | Heterodimerization Mincle/MCL | FcRγ–Syk         | Activating (81–84) |
|                  |             |           | Inhibitory ITAM                | FcRγ–Syk–SHP-1   | Inhibitory (91, 92) |
|                  |             |           | Sensing self                   | Retarded Syk     | Inhibitory (104, 105) |
|                  |             |           | Heterologous modulation        | FcRγ–Syk         | Activating (7, 100–103) |
|                  |             |           |                                | FcRγ–Syk–PKB–Mdm2| Activating (140, 141, 143) |
|                  |             |           |                                |                 | Inhibitory (146) |
| hemITAM          | CLEC-2      | CLEC1B    | Homodimerization               | Syk              | Intrinsic (75, 76) |
|                  | DNGR-1      | CLEC3A    | Motif context                  | Syk              | Intrinsic (19, 60–64) |
|                  | Dectin-1    | CLEC7A    | Subcellular location           | Syk              | Intrinsic (67–70) |
|                  |             |           | Ligand size-conditioned subcellular location | Syk–MAPK–reactive oxygen species | Inhibitory (93, 94) |
|                  |             |           | Phosphatase association        | SHP-1–PTEN–FcRγ SHIP-1 | Activating (85) |
|                  |             |           |                                | SHP-2–Syk        | Activating (124–126) |
|                  |             |           |                                | Pi3K–mTOR–HIF-1α | Activating (130–134) |
|                  |             |           |                                | Syk–Pyk2–EPK–SOCS-1 | Inhibitory (128) |
| ITIM             | DCIR        | CLEC4A, Clec4a2 | Activating ITIM | IFNI–STAT1 SHP-2 hijacking (?) | Activating (58) |
|                  |             |           | Sensing self and non-self      | SHP-2/SHIP-1     | Activating (57) |
|                  |             |           |                                |                 | Inhibitory (108, 109) |

* Described in the indicated reference. In case it was not studied in depth, it might be incomplete.

**This column indicates the inflammatory balance provided by each source of signaling flexibility. “Intrinsic” refers to specific responses triggered by particular CLRs.**
FIGURE 2 | Signaling flexibility downstream of C-type lectin receptors (CLRs). Signaling triggered downstream of CLRs goes beyond the canonical modules present in their intracellular domains and can be modulated by different processes. Some examples of such plasticity are represented. (A) DNGR-1 promotes cross-presentation of antigens to CD8+ T cells, yet not directly contributing to inflammation. (B) Mincle and MCL dimerize, boosting phagocytosis, and spleen tyrosine kinase (Syk)-mediated inflammatory responses. (C) Sensing of a soluble ligand from *Leishmania* by Mincle triggers an inhibitory immunoreceptor tyrosine-based activating motif conformation downstream of Fc receptor γ (FcRγ), where SHP-1 dampens inflammatory responses triggered by heterologous receptors. (D) The phosphatase SHP-2 acts as a scaffold downstream of Dectin-1 and FcRγ-coupled CLRs, facilitating the recruitment of Syk and its inflammatory signaling. (E) Both self and non-self ligands share signaling pathways downstream of DC-SIGN depending on whether they are mannosylated or fucosylated glucans.

The diagram illustrates the signaling pathways and molecules involved in the responses triggered downstream of different CLRs. The different signaling pathways and molecules are color-coded to highlight their interactions and pathways.

Receptor to adopt different conformations that depend on pH and ionic strength, modulating its function as the receptor progresses through the endocytic pathway (64). Even the inflammatory response of mouse and human Dectin-1 to the same ligand varies because of minor interspecies variations in the signaling motif, with low valency ligands inducing proinflammatory genes through human but not mouse Dectin-1 (65).

Receptor location also affects CLR signaling and functions. A single CLR may be expressed in different cell types (66) as diverse isoforms that may differ in subcellular location. For example, two isoforms of Dectin-1 have been described to bind β-glucans (67); isoform A is characterized by the presence of a stalk region including an N-linked glycosylation site, which is missing in isoform B (68). This glycosylation determines the cell surface expression of isoform A, while non-glycosylated isoform B is retained intracellularly, thus conditioning the response to ligands (69) and the sensitivity to proteolytic cleavage (70).

The subcellular location of a CLR may not only depend on intrinsic features in its sequence, but also on the size of the particle where the ligand is recognized. “Frustrated” phagocytosis mediated by Dectin-1 in response to ligands exposed in large particles leads to enhanced cytokine response and ROS production compared with soluble ligands (71–73). Blockade of Dectin-1 internalization following ligand exposure leads to sustained MAPK activation (72), suggesting that endocytosis dampens Dectin-1 production of cytokines. Thus, formation of a phagocytic synapse by particulate β-glucan redistributes Dectin-1 and phosphatases along the cellular membrane, favoring proinflammatory signals including ROS production (73). In addition, the size of the ligand-containing particle and the consequent location of the receptor, can lead to qualitatively different responses. Dectin-1-mediated phagocytosis dampens the nuclear translocation of neutrophil elastase, controlling the extent of neutrophil extracellular traps (NET) formation in response to small pathogens (bacteria or yeast). Consequently, Dectin-1 blockade or deficiency leads to enhanced NETosis, as observed in response to non-phagocytic large pathogens (hyphae) (74).

Thus, the expected canonical response based on signaling modules can be altered both by slight modifications in motif context and the subcellular location of CLRs, taking into account that the latter may be affected by the size of the ligand recognized.

**Multimerization of CLRs for Signaling**

The signal transduction through several myeloid CLRs may also depend on their capacity to form dimers or multimers with other CLRs. CLRs bearing hemITAMs may require two phosphorylated tyrosines in a homodimer to bind Syk. It has been shown that CLEC-2 preexists as a dimer that aggregates following ligand binding (75, 76). The hemITAM motif of CLEC-2 is crucial for blood-lymph separation during development (77, 78). Of note, thrombus stability is dependent on CLEC-2 but not on the hemITAM, revealing a hemITAM-independent signaling for CLEC-2 (79).

DC-SIGN provides another example of homomultimerization, despite lacking ITAM or ITIM domains. This CLR appears assembled as a tetramer, allowing multiple interactions with diverse pathogens that differ in size, but also increasing ligand avidity (80). In addition, some CLRs form heterodimers, such as MCL and Mincle (11, 81). These two CLRs are interrelated as they both sense the mycobacterial glycolipid trehalose-6,6-dimycolate.
(TDM), triggering an FcRγ-dependent pathway (11). Indeed, MCL and Mincle are coregulated and depend on each other for their mutual surface expression (82, 83). However, the association of MCL with FcRγ in this complex is species-specific, being direct in mouse cells (11) but requiring Mincle in rat (81). Thus, the interaction between these CLRs would facilitate MCL signaling capacity via association with Mincle and translocation to the plasma membrane. On the other hand, Mincle would benefit the endocytic capacity of MCL (Figure 2B) and both receptors could increase affinity or specificity for their ligands (84). MCL also forms a heterodimeric pattern-recognition receptor with Dectin-2 (85), which has a high affinity for α-mannans on the surface of Candida albicans (C. albicans) hyphae.

Cooperative interaction is also found in the case of dengue virus binding with high affinity to MR and DC-SIGN, receptors that subsequently handle the virus to the lower affinity receptor CLEC5A, which mediates signal transduction (86).

All these examples illustrate how multimerization of CLRs, forming either homo- or hetero-complexes, facilitates a cooperative response to the ligand.

Is the Function of CLRs Inhibitory or Activating?

Another layer of complexity in CLR signaling stems from the ability of a single CLR to bind different ligands through its plastic C-type lectin domain. For instance, depending on their relative affinity or avidity, ligands may fine-tune signaling pathways downstream of ITAM motifs. Whereas the binding of high-avidity ligands to these receptors induces activating signals, the binding of low-avidity ligands leads to hypophosphorylation of the ITAM domain and preferential association of SH2-containing phosphatases like SHP-1, a configuration known as “inhibitory ITAM” (87). Although FcγRI receptor, which associates for signaling with the FcRγ chain, is the paradigmatic example of this inhibitory pathway (88–90), we have shown that CLRs associated with FcRγ chain may behave in the same fashion.

As an example, Mincle senses a soluble ligand derived from Leishmania that induces phosphorylation of SHP-1 coupled to FcRγ chain, inhibiting DC activation through heterologous receptors (Figure 2C) (91). In addition, SHP-1 contributes to deceleration of phagosome maturation upon TDM binding, suggesting an inhibitory signal downstream of Mincle during phagocytic processes (92). MR binds the FcRγ chain and, upon sensing Mycobacterium tuberculosis, recruits SHP-1 to the phagosome, thus limiting PI(3)P generation and delaying fusion with the lysosome, which promotes M. tuberculosis growth (12). Following treatment of DCs with curdlan or depleted zymosan (lacking TLR-stimulating properties), Dectin-1 signaling is modulated by the association of SHP-1 and PTEN to the FcRγ chain, hindering cytokine expression, DC maturation, and T-cell proliferation (93). ROS production downstream of Dectin-1 sensing of C. albicans is also tightly regulated by the SH2-domain containing inositol 5’ phosphatase (SHIP)-1 in response to Dectin-1 ligands (94). Thus, association of phosphatases to “activating” CLRs depending on the ligand nature, binding affinity, or avidity may contribute to maintenance of immune homeostasis.

Conversely, tyrosine phosphatases can contribute to activation. Contrary to SHP-1, the related tyrosine phosphatase SHP-2 acts as a scaffold, facilitating the recruitment of Syk to Dectin-1 or the adaptor FcRγ chain (95) (Figure 2D). In this way, DC-derived SHP-2 was crucial in vivo for the induction of TNF-α, IL-6, IL-12, and Th1 and Th17 anti-fungal responses upon C. albicans infection (95).

Immunoreceptor tyrosine-based inhibitory motif-coupled receptors can also deliver an activating signal. In a model of tuberculosis infection in non-human primates, DCIR deficiency impairs STATI-mediated type I IFN signaling in DCs, leading to increased production of IL-12 and differentiation of T lymphocytes toward Th1. Thus, DCIR-deficient mice with increased Th1 immunity control M. tuberculosis better than WT animals, but also shown increased inflammation in the lungs mediated by TNF-α and inducible nitric oxide synthase (iNOS) (58). This study suggests that DCIR acts as an activating receptor for the STATI-type I IFN signaling, and speculates that DCIR may function as a molecular sink binding unphosphorylated inactive SHP-2, therefore, limiting SHP-2’s capacity to deactivate STAT1.

The examples explained above illustrate a lack of correspondence between the canonical motif coupled to a CLR and the resulting signaling pathway. Association to kinases would lead to activating routes, while association to phosphatases would result in regulatory pathways, with some exceptions like the SHP-2-mediated CLR-induced activation (95). Association of kinases or phosphatases could be related to the strength of the initiating signal, with suboptimal phosphorylation leading to phosphatase binding to the hypo-phosphorylated ITAM (inhibitory ITAM) (87). Due to the signaling flexibility offered by CLRs, a detailed empiric analysis for each CLR-ligand interaction in terms of type of ligand, concentration, and kinetics of exposition would be required to predict the signaling outcome.

Dealing With Self and Non-Self

C-type lectin receptors act as plastic receptors, some of them detecting self-ligands, other detecting non-self ligands, and many of them acting as dual receptors sensing self and non-self. It is possible that CLRs will behave as activating receptors when they sense non-self ligands, while CLRs bearing an ITIM motif will preferably bind self to dampen inflammation. However, CLRs bearing an ITAM motif will preferentially bind to viral components. Thus, in opposition to non-dangerous self, also known as “self-associated molecular patterns” (96, 97), Polly Matzinger proposed the existence of dangerous-self (damage-associated molecular patterns or DAMPs) exposed and/or released upon necrotic cell death (98, 99). In addition, tissue damage signals concomitant to infection can contribute to effector responses. Thus, DNGR-1 senses tissue damage concomitant with viral infections and facilitates antigen processing of viral antigens for cross-presentation to CD8+ T cells, decoding the antigenicity rather than the adjuvanticity of the cargo (60–63). Some examples of CLRs dealing with self and non-self ligands are explained below.

Mincle is a plastic CLR promoting proinflammatory signals after sensing glycolipids in the cell wall of bacteria and fungi (24–26), but also sensing damaged self in the form of soluble SAP-130 following necrosis (7). Mincle sensing of β-glucosylceramide
to DC-SIGN as a dual receptor that, depending on the nature of the ligand, contributes to maintain homeostasis or initiates the immune response against some pathogens.

All these examples illustrate how a single CLR can trigger different signaling pathways depending on the recognition of self or non-self ligands. Current understanding of these processes is based on the study of individual CLRs. Deciphering common signaling patterns for self versus non-self sensing would allow harnessing immunity and inflammation by CLRs.

MODULATION OF HETEROLOGOUS SIGNALING BY MYELOID CLRs

In addition to the diverse response of a single CLR depending on the stimulus, it is fascinating how these signaling pathways interact with signals from heterologous receptors and lead to complex responses to stimuli that are simultaneously detected by several myeloid PRRs expressed in myeloid cells [see also Ref. (116, 117) for reviews focused on this topic]. In this section, we illustrate some examples of how myeloid CLRs cross-talk with surrounding heterologous receptors.

Dectin-1 Affects Simultaneous and Deferred Signaling Through Heterologous Receptors

Dectin-1 triggers a response after sensing infectious agents, such as diverse fungi and mycobacteria (118), Salmonella typhimurium (119) or Leishmania infantum (120). Dectin-1 may also promote proinflammatory signals following the detection of endogenous factors, such as vimentin from atherosclerotic plaques (121), galectin-9 from pancreatic carcinoma (122), or N-glucans on tumor cells (123). In addition to a prototypical activating CLR, Dectin-1 modulates signals simultaneously triggered through other PRRs. Dectin-1 cooperates with signals from TLR2/MyD88 to increase proinflammatory cytokine production (124–126). This synergy is exerted at the level of effector responses resulting in increased production of TNF-α, IL-12, and ROS (124) (Figure 3A, left). Dectin-1 also positively cooperates in the full activation of the NLRP3 inflammasome, participating in the priming and generation of pro–IL-1β and the induction of ROS required for NLRP3 activation (127).

Conversely, Dectin-1 stimulation with depleted zymosan in bone marrow macrophages leads to Syk and Pyk2-Erk-dependent activation of SOCS-1 that downregulates IL-10 and IL-12p40 production induced by TLR9 stimulation (128) (Figure 3A, right). This effect would contribute to the Dectin-1 signature in priming Th17 responses (40, 128). In addition, Dectin-1 protects against chronic liver disease by suppressing TLR4 signaling. This effect is mediated by reducing TLR4 and CD14 expression, which are regulated by Dectin-1-dependent macrophage colony stimulating factor expression (129).

Apart from direct modulation of signaling pathways triggered simultaneously, Dectin-1 can leave a footprint that affects deferred signaling by heterologous receptors, a process named as trained immunity (130). Trained immunity after sensing of C. albicans or purified β-glucan via Dectin-1 results in enhanced protection to a lethal challenge with Candida and cross-protection...
to *Staphylococcus aureus* infection (130, 131). This increased protection upon a later infection is linked to increased proinflammatory responses to delayed rechallenge with different TLR ligands, such as LPS or Pam3Cys4 (130) (Figure 3B), or bacteria, i.e., *Bacteroides fragilis*, *Escherichia coli*, *Staphylococcus aureus*, *Borrelia burgdorferi*, or *M. tuberculosis* (130, 132, 133).

In monocytes, Dectin-1 signaling triggers the PI3K-Akt pathway, leading to activation of mTOR and HIF-1α (131). This leads to a shift from oxidative phosphorylation to aerobic glycolysis. Accumulation of fumarate, associated with glutamine replenishment of the TCA cycle, inhibits KDM5 histone demethylases, a key step for induction of monocyte epigenetic reprogramming that underlies the long-lasting effects of trained immunity (130, 134) (Figure 3B).

Apart from β-glucan or *Candida*, several other self and non-self ligands, such as chitin (135), BCG vaccine (136), and uric acid (137) induce trained immunity (137, 138). It would thus not be surprising that more CLRs could contribute to trained immunity. In this regard, although *C. albicans* mannans, potentially sensed by MR, Dectin-2, or Mincle (46), have shown not to prime human monocytes directly (130), they are essential for *C. albicans*-induced training (133). Furthermore, both Dectin-1 and MR are needed to trigger glycolysis upon *C. albicans* stimulation (139); this glycolytic switch constitutes a critical metabolic
step in trained immunity induction (131, 139). Trained immunity triggered by Dectin-1 and potentially other CLRs is thus a consequence of metabolic switch and epigenetic programming that affects deferred heterologous signaling.

**Mincle-Triggered Regulatory Responses**

As described before, Mincle triggers an FcR y-mediated activating signal in response to different stimuli. In addition, Mincle engagement can deliver regulatory responses affecting signaling pathways triggered by heterologous PRRs, such as TLRs or other CLRs, for example, Dectin-1. This section will explore modulation of heterologous receptors by Mincle.

Mincle is induced following TLR activation (7). Following sensing of *Fonsecaea pedrosoi*, Mincle triggers an incomplete inflammatory response that requires synergistic TLR stimulation to induce a potent proinflammatory response (Figure 3C, left), needed to clear the infection in a mouse model of chromoblastomycosis (140). This cooperative activation through Mincle and TLRs is particularly effective in human newborn DCs. Co-stimulation using the Mincle agonist trehalose-6,6-dibehenate and the TLR7/8 agonist R848 led to enhanced caspase-1 and NF-kB activation, Th1 polarizing cytokine production and autologous Th1 polarization (141).

However, Mincle exhibits a dual role in promotion and subsequent resolution of inflammation. Mycobacteria express ligands for TLRs which induce expression of Mincle that can then detect TDM and contribute to inflammation. Mincle via the Syk/p38 axis can also lead to eIF5A hypusination that increases translation efficiency of iNOS, which is transcriptionally induced by TLR2 ligation (142). In this way, Mincle favors NO production that inhibits late-stage activation of NLRP3 inflammasome in TDM-induced inflammation, contributing to termination (142). Similarly, TLR2 sensing of Corynebacterium induces robust Mincle expression, which cooperatively detects corynebacterial glycolipids favoring production of granulocyte colony stimulating factor and NO (143).

Dectin-1 and Mincle are involved in the recognition of *Fonsecaea monophora*, a pleomorphic fungus also responsible for chromoblastomycosis (144, 145). Signaling triggered by Dectin-1 initiates protective immunity against the fungus by activating IRF1 and IL-12p35 transcription. However, these responses are dampened by the Mincle/Syk axis, in a process involving PI3K/PKB-mediated activation of the E3 ubiquitin ligase Mdm2, leading to degradation of IRF1 and repression of IL-12p35 production (Figure 3C, right). In this way, Mincle sensing of *F. monophora* dampens induction of protective Th1 immunity triggered by Dectin-1 (146). Mincle is also targeted by Leishmania parasites to evade the priming of Th1 immunity initiated by DCs. As explained above, Mincle recruits SHP-1 to an inhibitory ITAM configuration in the coupled FcR y chain, and this results in inhibition of DC activation by heterologous receptors sensing Leishmania or LPS (91) (Figure 2C). Mincle ligation can also reduce TLR4-mediated inflammation, whereas Mincle deletion or knockdown results in exaggerated inflammation in response to LPS. This effect is mediated through the control of TLR4 coreceptor CD14 expression (147).

**Tailoring Immunity Through DC-SIGN**

DC-SIGN engagement does not generally induce the expression of cytokines by itself, but rather modulates responses initiated by TLRs. Thus, glycans from the helminth *Fasciola hepatica* are recognized by DC-SIGN leading to enhanced TLR-induced IL-10 and IL-27p28, triggering a tolerogenic program that differentiates naïve CD4+ T cells into regulatory T cells (148) (Figure 3D, left). However, the interaction of DC-SIGN with the salivary protein Salp15 from the tick *Ixodes scapularis* dampens inflammatory responses triggered by *Borrelia burgdorferi*. Raf-1 activation downstream of DC-SIGN sensing Salp15 results in MEK-dependent decrease of IL-6 and TNF mRNA stability and impaired nucleosome remodeling at the IL-12p35 promoter, modulating TLR-induced DC activation and T cell proliferation (112) (Figure 3D, right).

All these examples clearly illustrate how signaling pathways triggered by CLRs can have an impact on responses mediated by surrounding heterologous receptors, adding an extra layer of complexity to our understanding of CLR-mediated responses.

**CONCLUDING REMARKS**

Classical sorting of myeloid CLRs based on the structure of the C-type lectin domain does not have functional significance. A more recent classification based on the presence of ITAM, hemITAM, or ITIM intracellular signaling motifs associated with the receptors has been useful as a starting point to predict the functional outcome of signaling CLRs (1). However, many factors may alter the expected canonical response. Minor variations in the context of the canonical motifs result in different signaling and effector outcomes (60, 65). Subcellular location depending on the isoform (69) or conformation of the receptor based on specific residues (64) also affects the function of the receptor. CLR signaling also depends on the size of the particle, where the ligand is recognized, affecting quantitatively the strength of the reaction (71–73) and also leading to qualitatively different responses (74, 149). Cooperative binding and signal transduction may be a consequence of multimerization. There are examples of homodimerization (75, 76) and formation of hetero-complexes (11, 81, 84–86). Hetero-complexes result in a mutual benefit for involved receptors, combining avidity for the ligand, capacity for endocytosis and/or signal transduction capabilities.

The plasticity of the C-type lectin domain allows binding to different ligands that, depending on their relative affinity or avidity, may trigger activating or inhibitory signaling pathways downstream of the same motifs. For example, low-avidity ligands drive a Syk-dependent association with SHP-1 to the ITAM domain (87, 88, 90), with a growing list of examples illustrating CLRs coupled to the FcR y chain (12, 91–93). Conversely, tyrosine phosphatases may contribute to activation (95) and ITIM-containing CLRs may trigger activating signals (58). These results evidence the fine regulation of signaling though a single receptor based on differential interaction with diverse ligands, leading to the hypothesis that sensing self-ligands through CLRs could drive tolerance while non-self ligands could provoke immunity. However, dangerous-self could rather contribute to immunity and some non-self ligands could inhibit immune response for...
evasion, making the final outcome of a single response rather unpredictable. In addition, the concerted sensing of complex ligands by a variety of PRRs leads to complex integrated responses. CLRs may affect signals of heterologous receptors that are simultaneously triggered, either enhancing or modulating the response (59, 91, 115, 124–126, 128, 142, 146). Of note, Dectin-1 induces a metabolic switch and epigenetic programming that affects deferred heterologous signaling (130, 131). In conclusion, understanding how different signaling pathways triggered by CLRs and heterologous receptors act in concert during sensing self and non-self remain a fascinating endeavor.

Research in the field of CLRs has gained much attention considering the diversity of members, ligands, expression pattern on clinically relevant cellular populations and their relevant function on the initiation, and regulation of immunity and inflammation. Some of these features have been illustrated here and offer multiple possibilities to harness CLR-triggered responses. However, CLR manipulation may lead to unexpected outcomes and needs to be tested empirically. In addition, deciphering molecular signatures common to signaling pathways triggered by CLRs in response to different ligands will help to understand their precise role in immunity and inflammation.

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CF, SI, PS-L, MM-L, and DS conceived and wrote the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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