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Immunobiology of C-Type Lectin Receptors

Susanne Roth[a] · Christina Thomas[a] · Jürgen Ruland[a, b]

[a]Institut für Klinische Chemie und Pathobiochemie, Klinikum rechts der Isar, Technische Universität München, Munich, and [b]Laboratory of Signaling in the Immune System, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany

Abstract

C-type lectin receptors (CLRs) that signal via the kinase Syk are an important class of pattern recognition receptors in the innate immune system. They recognize pathogen- and host-derived danger signals, and are best known for their role in antifungal immunity. Here, we review recent insights into the molecular mechanisms of CLR signaling, and their significance in host defense.

Cells of the innate immune system such as dendritic cells, macrophages and neutrophils are equipped with a wide array of germline-encoded pattern recognition receptors (PRRs) that are essential for host protection and tissue homeostasis [1]. These include the transmembrane Toll-like receptors and C-type lectin receptors (CLRs), as well as cytosolic sensors of the nucleotide-binding oligomerization domain-like receptor and retinoic acid-inducible gene-I-like helicase families [2, 3]. PRRs detect not only pathogen-associated molecular patterns derived from viruses, bacteria, or fungi, but also endogenous damage-associated molecular patterns, which are released upon tissue injury. Upon sensing their cognate ligands, PRRs engage distinct intracellular signaling pathways that ultimately allow myeloid cells to elicit inflammation and shape adaptive immune responses. One key signaling cascade that is crucial for inflammatory responses, including those initiated by CLRs, is the NF-κB signaling pathway [4].

Danger Recognition by C-Type Lectin Receptors

This discussion focuses on a specific sub-family of CLRs that are characterized by their ability to recruit and activate the tyrosine kinase Syk [5–7]. Dectin-1 is the prototypic Syk-activating CLR, and the first CLR identified to be essential for host defense [5–7]. It is known for its role in antifungal immunity, and possesses an extracellular C-type lectin domain that recognizes β-glucans in fungal cell walls [7]. The intracellular signaling domain of dectin-1 contains an immunoreceptor tyrosine-based activation motif (ITAM)-like motif [2, 6]. Upon ligand binding, the ITAM is phosphorylated by Src family tyrosine kinases, thereby creating a docking site for the recruitment and activation of Syk [2, 3, 6]. Other CLRs that are involved in anti-fungal immunity are dectin-2

and mincle [8–11]. However, in contrast to dectin-1, dectin-2 and mincle do not possess ITAMs themselves, but instead associate with the ITAM-containing signaling adapter Fc receptor-γ chain for Syk activation [6, 10, 11].

In addition to fungi, CLRs can also detect microbial pathogens and endogenous ligands. Mincle, for example, has been recently identified as the activating receptor that recognizes the mycobacterial cord factor, trehalose-6,6-dimycolate [12, 13], and also the self-ligand SAP130 that is released by necrotic cells [14]. The ITAM-containing CLR CLEC9a is a danger receptor for the recognition of endogenous signals released upon cellular damage [15]. Recent work has identified the ligands for CLEC9a to be cytoskeletal components [16, 17]. Interestingly, recognition of non-fungal ligands by CLRs is not always protective. Dectin-2 can, in addition to fungi, also recognize the helminth *Schistosoma mansoni* [18]. However, it is thought that signaling via dectin-2 during schistosomal infection contributes to immunopathology [18]. Similarly, recognition of dengue virus by CLEC5a, which signals via Syk by using the ITAM-containing adapter protein DAP-12, is required for lethal disease caused by this virus [19].

C-Type Lectin Receptor Effector Pathways

Syk activation by CLRs drives several cellular responses, including phagocytosis of fungal particles, production of microbicidal reactive oxygen species (ROS), as well as altered gene expression [6]. Activation of NF-κB signaling is a critical event downstream of numerous Syk-coupled CLRs, and the ability of these receptors to activate NF-κB signaling requires the adapter protein Card9 [6, 20, 21]. Card9 possesses a caspase recruitment domain (CARD) and a coiled-coil region, and is a myeloid cell-specific member of a small family of CARD-coiled-coil proteins which also includes Card10, Card11 and Card14 [22]. Initial work demonstrated that Card9-deficient mice are highly susceptible to infection with the opportunistic fungal pathogen *Candida albicans*, and that Card9 is critically required for cytokine production upon dectin-1 stimulation [20, 21]. The requirement of Card9 for anti-fungal immunity is also reflected in humans, where a homozygous loss-of-function mutation in CARD9 results in high susceptibility to fungal infections [23]. However, in the context of infection, Card9 probably integrates signals not only from dectin-1, but also from dectin-2 [8] and mincle [13, 24]. Thus, although there is some redundancy at the receptor level, Card9 represents a non-redundant factor critical for anti-fungal defense.

Molecularly, Card9 cooperates with the adapter protein Bcl10 and the paracaspase Malt1 to selectively transduce signals from Syk to the canonical IKK-dependent NF-κB pathway. Signaling via the Card9, Bcl10, Malt1 complex operates independently from CLR-induced ROS production and phagocytosis [6]. Recent findings have given insight into the mechanisms of how Syk-coupled CLRs activate the Card9 complex. Stimulation of innate immune cells with CLR ligands induces Syk-dependent phosphorylation and activation of the serine/threonine kinase PKCδ [25]. PKCδ then phosphorylates Card9 at Thr231, which is required for the signal-induced association of Card9 with Bcl10 and Malt1, and the subsequent recruitment of TAK1 for activation of the canonical NF-κB pathway. Consistently, PKCδ-deficient dendritic cells are defective in innate immune responses to dectin-1, dectin-2 or mincle stimulation, and PKCδ-deficient mice are highly susceptible to fungal infection [25].

The proinflammatory cytokine interleukin-1β (IL-1β) is critical for host defense against fungal infection [26]. The production of IL-1β requires NF-κB-mediated upregulation of pro-IL-1β, and subsequent proteolytic conversion of pro-IL-1β to the bioactive and secreted form. The latter event is typically mediated by caspase 1

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within the context of cytosolic complexes termed inflammasomes [27]. Upon cellular infection with *C. albicans*, CLR-induced Syk signaling is required for both pro-IL-1β synthesis and activation of the Nlrp3 inflammasome [28]. While pro-IL-1β synthesis selectively requires the Card9 pathway, inflammasome activation by fungi involves ROS production and potassium efflux. Activation of the Nlrp3 inflammasome downstream of CLRs is required for anti-fungal immunity, since Nlrp3-deficient mice are highly susceptible to *C. albicans* infection [28]. Thus, there is a cross talk between Syk-coupled CLRs and Nlrp3, at least in the context of fungal infection. However, the exact mechanism of how CLR signaling couples to the inflammasome remains uncharacterized.

Conclusions

Syk-coupled CLRs play a broad role in innate immunity and can also couple innate to adaptive immune responses. Recent studies have uncovered critical signaling molecules downstream of CLRs, such as Syk, Card9 and PKCδ. Most work has focused on the role of CLR and Card9 signaling in anti-fungal defense. However, Card9-deficient mice are also highly susceptible to *Mycobacterium tuberculosis* infection [29], presumably due to the critical role of Card9 downstream of the cord factor receptor mincle [13, 24]. Thus, additional work is required to further characterize the signaling mechanisms of CLRs, and their role in other infections and sterile tissue damage. These studies will provide important insights into the molecular regulation of host defense and the control of tissue homeostasis.

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Prof. Jürgen Ruland
Klinikum r.d. Isar
Institute for Immunology
Schneckenburgerstrasse 8
DE–81675 Munich (Germany)
E-Mail jruland@lrz.tu-muenchen.de

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