Review

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Caspase recruitment domain-containing protein 9 signaling in innate immunity and inflammation

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Caspase recruitment domain-containing protein (Card)9 is a nonredundant adapter protein that functions in the innate immune system in the assembly of multifunctional signaling complexes. Together with B cell lymphoma (Bcl)10 and the paracaspase, mucosa-associated lymphoid tissue lymphoma translocation protein (Malt)1, Card9 links spleen-tyrosine kinase (Syk)-coupled C-type lectin receptors to inflammatory responses. Card9 signaling also responds to intracellular danger sensors, such as retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs) and nucleotide-oligomerization domain (Nod)2. Card9 complexes are engaged upon fungal, bacterial, or viral recognition, and they are essential for host protection. Moreover, Card9 polymorphisms are commonly associated with human inflammatory diseases. Here, we discuss the molecular regulation and the physiological functions of Card9 in host defense and immune homeostasis, and provide a framework for the therapeutic targeting of Card9 signaling in immune-mediated diseases.

Pattern recognition receptors (PRRs)

Innate immune system cells, including macrophages and dendritic cells, express various germline-encoded PRRs that detect microbial structures (pathogen-associated molecular patterns; PAMPs) upon infection or endogenous danger molecules (danger-associated molecular patterns; DAMPs) following cell injury [1,2]. PRRs with signaling capacity include Toll-like receptors (TLRs), Nod-like receptors (NLRs), RLRs, and spleen-tyrosine kinase (Syk)-coupled C-type lectin receptors (CLRs) [2–5]. Following receptor ligation, these PRRs engage a limited number of intracellular signaling modules that integrate information and connect danger recognition to immune effector function. One central adapter molecule that assembles such signalosomes is Card9. Card9 can associate with the adaptor protein Bcl10 and the paracaspase Malt1 to relay PRR signals to key transcription factors for the orchestration of acute inflammatory responses and for the subsequent initiation of adaptive immunity [6,7]. Recent work has demonstrated that these conserved pathways are essential for host protection in humans and mice [8–14]. Still, it is unclear how Card9–Bcl10–Malt1 (CBM) signalosomes are activated on a molecular level and how CBM responses are transduced to effector cascades. Moreover, although polymorphisms in the Card9 gene were recently detected in human inflammatory diseases such as Crohn's disease (CD) or ankylosing spondylitis (AS) [15–19], the pathophysiological roles of Card9 signaling in complex disorders are unknown. This review discusses the molecular mechanisms of Card9 signaling, together with the physiological and potential pathological roles of Card9 signaling during innate immune responses *in vivo* to open perspectives for a therapeutic manipulation of the respective pathways.

Card9 structure, expression, and biochemistry

Card9 was originally identified via a database search for caspase-associated recruitment domain (CARD) proteins [20]. CARDs are protein–protein interaction modules that mediate homophilic associations between CARD-containing molecules. Card9 possesses, in addition to the N-terminal CARD, a coiled-coil region at the C terminus that serves as an oligomerization domain (Figure 1). The human and the mouse proteins share 83% identity and about 87% similarity. The architecture of Card9 is similar to the architecture of the CARD-containing membrane-associated guanylate kinase (MAGUK) (Carma) family of proteins, which includes Carma1 [also known as Card11 and Bcl10-interacting MAGUK protein (Bimp)3], Carma2 (also known as Card14 and Bimp2), and Carma3 (also known as Card10 and Bimp1). However, Card9 does not possess the C-terminal MAGUK motif that is characteristic of Carma proteins and is involved in membrane association. Card9 expression has been detected in the thymus, spleen, lung, liver, placenta, brain, bone marrow, and peripheral blood. At the cellular level, Card9 is highly expressed by myeloid cells, particularly antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages [7,9,20,21].

Initial studies have demonstrated that Card9 directly binds to Bcl10 via CARD–CARD interaction [20]. Bcl10 is a signaling adapter that mediates nuclear factor (NF)-κB activation in response to multiple upstream stimuli and triggers the activation of mitogen-activated protein kinases (MAPKs), c-Jun N-terminal kinase (JNK), and p38 [6,7,22]. Bcl10 directly binds to the paracaspase Malt1,

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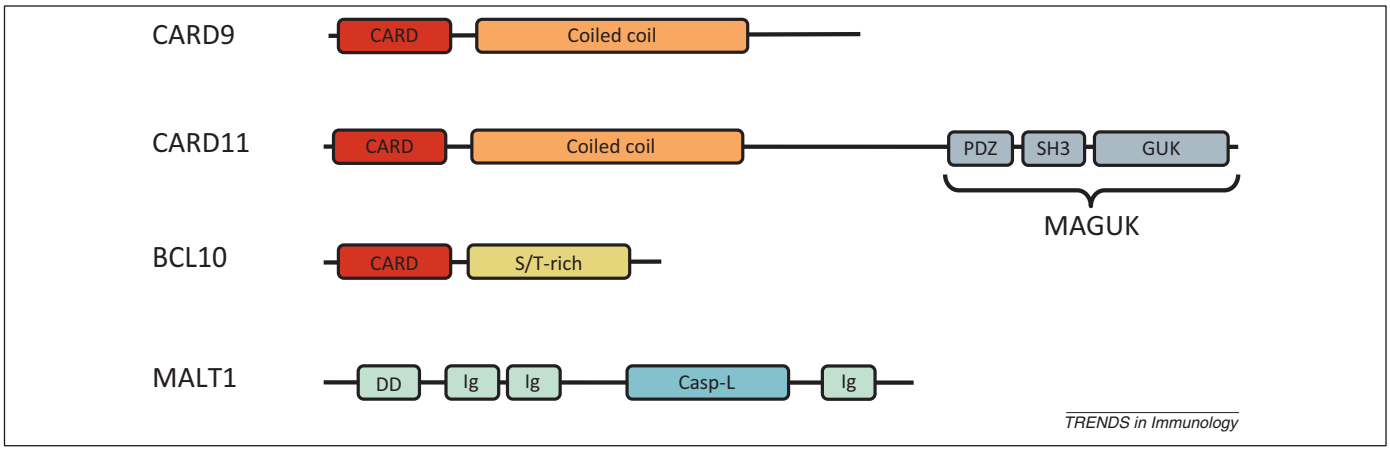

Figure 1. Caspase recruitment domain-containing protein (Card)9, Card11, B cell lymphoma (Bcl)10, and mucosa-associated lymphoid tissue lymphoma translocation protein (Malt)1 protein structure. Card9 contains a caspase-associated recruitment domain (CARD) and a coiled-coil domain, as in Card11, which also possesses a membrane-associated guanylate kinase (MAGUK) region composed of a PSD95, DLGA, and ZO1 homology (PDZ), Src-homology 3 (SH3), and guanylate kinase (GUK) domain. Bcl10 also contains an N-terminal CARD, which binds to the CARDs of Card9 and Card11. The serine and threonine (S/T)-rich region of the Bcl10 molecule plays an inhibitory role in signal transduction. The paracaspase Malt1 contains a proteolytically active central caspase-like (Casp-L) domain and several protein–protein interaction motifs, including an N-terminal death domain (DD), two immunoglobulin (Ig)-like domains and a third C-terminal Ig-like domain.

inducing cell activation. Malt1 is composed of an N-terminal death domain (DD), immunoglobulin (Ig)-like domains, and a caspase-like region [23] (Figure 1). The caspase-like domain of Malt1 possesses proteolytic activity [24–27]. In contrast to classic caspases, Malt1 mediates specific cleavage of substrates adjacent to positively charged arginine residues instead of negatively charged aspartic acid [28]. In addition to its enzymatic activity, Malt1 possesses scaffolding functions required for the recruitment of factors such as tumor necrosis factor (TNF) receptor associated factor (TRAF)2 and TRAF6 for the engagement of canonical inhibitor of NF-κB (IkB) kinase (IKK)-dependent NF-κB signaling. TRAF6 catalyzes the lysine-63 (K63) polyubiquitination of Bcl10 and Malt1, thereby providing a docking site for IKKγ and recruiting the IKK complex. The proteolytic activity of Malt1 inactivates negative regulators of NF-κB or MAPK signaling, such as A20 or cylindromatosis (Cyld) protein, and thereby fine-tunes or prolongs cell-activating signals under certain conditions [23,28]. The signal-induced association of Card9, Bcl10, and Malt1 results in the formation of signalosomes, which are also known as the innate or myeloid CBM complexes.

Molecular function of CBM complexes in Dectin-1 signaling

Initial genetic studies in mice have shown that Card9 couples signals from immunoreceptor tyrosine-based activation motif (ITAM) receptors to the canonical NF-κB pathway [10,29]. The ITAM signaling motif was originally identified in T and B cell antigen receptors and provides the docking site for tandem Src homology 2 (SH2) domain-containing spleen tyrosine kinase family members [Syk Zeta-chain-associated protein kinase (ZAP)-70] for Syk/ZAP-70 recruitment and activation. Innate immune cells express a variety of ITAM-containing or ITAM-coupled receptors on the cell surface, including PRR members of the CLR family [3–5,7,22]. The prototype of signaling CLRs is the antifungal immune receptor Dectin-1 [30,31] (Figure 2). However, its ITAM-like motif does not correspond exactly to the classical ITAM sequence and includes only a single YxxL motif (also called hemITAM) per receptor molecule for Syk activation, in contrast to the tandem tyrosines present in the consensus sequence [3–5,7].

The recognition of fungal particles via Dectin-1–Syk signaling drives several cellular effector responses. It mediates the phagocytosis of fungal particles, controls the generation of reactive oxygen species (ROS), and reprograms gene expression for the production of inflammatory cytokines and chemokines (Figure 2). Card9-deficient DCs are defective in Dectin-1-induced NF-κB signaling. Dectin-1 specifically recognizes β-1,3 and β-1,6 linked β-glucan carbohydrates that are present in fungal cell walls and highly enriched in the fungal cell wall preparation zymosan. Consistently, murine Card9-deficient DCs are severely impaired in *Candida albicans*- and zymosan-induced cytokine production [10,29,32]. In addition, the purified β-glucan, curdlan, a selective Dectin-1 agonist [33], induces inflammatory responses in murine myeloid cells in a strictly Card9-dependent manner [33]. One study has indicated cell type-specific roles for Card9 in Dectin-1-mediated NF-κB activation [32]. Unlike myeloid differentiation primary response protein 88 (MyD88)-mediated NF-κB activation downstream of TLRs, which seems to operate cell type independently, Card9-mediated NF-κB activation downstream of Dectin-1 can vary between different cell types. In murine bone marrow-derived macrophages (BMDMs), thioglycolate-elicited peritoneal macrophages, and FMS-like tyrosine kinase 3 (Flt3) ligand (Flt3L)-derived DCs, Dectin-1–Card9 signals are insufficient to activate NF-κB and induce TNF production. By contrast, bone marrow-derived dendritic cells (BMDCs), resident peritoneal cells, and alveolar macrophages readily synthesize TNF upon Dectin-1 ligation [32]. However, the prestimulation of BMDMs with granulocyte–macrophage colony-stimulating factor (GM-CSF) or interferon (IFN)γ permits Dectin-1–Card9-mediated cytokine production, indicating that unknown cofactors may determine the cell type-specific regulation of Card9 signaling.

Dectin-1 stimulation leads to the Syk-mediated association of Card9 with Bcl10 and Malt1 and the formation of