CARD-Bcl10-Malt1 Signalosomes: Missing Link to NF-κB

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Cytokines, pathogens, and antigens signal through NF-κB (nuclear factor κB) transcription factors to regulate the expression of genes that mediate the inflammatory response. Stimulation of G protein–coupled receptors (GPCRs) has also been shown to trigger inflammatory responses by activating the canonical NF-κB pathway, which involves the phosphorylation and degradation of cytosolic NF-κB inhibitors (IκBs) that depend on the IκB kinase (IKK). However, genetic data about the molecular links between GPCR-proximal events and activation of IKK and, thereby, of NF-κB have remained elusive. Recent studies by Klemm *et al*., McAllister-Lucas *et al*., and Wang *et al*. now present compelling evidence that signaling complexes containing the adaptor proteins Bcl10 (B cell chronic lymphocytic leukemia and/or lymphoma 10) and Malt1 [mucosa-associated lymphoid tissue (MALT) lymphoma translocation gene 1], constitute the missing link between GPCRs and IKK and NF-κB activation (*1*–*3*). A separate study by Gross *et al*. reports an intriguing finding, that Dectin-1–mediated antifungal immunity in dendritic cells is also regulated by the Bcl10-Malt1 module (*4*). GPCR- and Dectin-1–induced NF-κB activation depends on the interaction of the Bcl10-Malt1 module with specific Bcl10-binding CARD (caspase recruitment domain)–containing scaffold proteins (*2*, *4*). Together with previous data establishing a crucial role for CARD11 [also known as CARMA1, for CARD-MAGUK (membrane-associated guanylate kinase)] association with Bcl10-Malt1 in lymphocytes, these results suggest that diverse receptor systems use distinct CARD scaffolds and conserved Bcl10-Malt1 modules to stimulate IKK and NF-κB signaling (Fig. 1).

CARD11-Bcl10-Malt1 Signalosome Links Antigen Engagement to NF-κB

Bcl10-Malt1 complexes were initially shown to link antigen receptor signaling in lymphocytes to downstream IKK and NF-κB activation (*5*, *6*). Engagement of an antigen with T or B cell receptors (TCRs or BCRs) triggers phosphorylation of CARD11 by protein kinase C (PKCθ in T cells; PKCβ in B cells). Phosphorylation of CARD11 induces a conformational change that permits recruitment of Bcl10-Malt1 to CARD11 and thereby directs assembly of the C11-B-M signalosome (CARD11-Bcl10-Malt1) (*7*, *8*). Complex formation requires the heterotypic interaction between the CARDs of CARD11 and Bcl10. Further, the CARD11 C-terminal MAGUK region is required for antigen-mediated NF-κB activation and anchors the C11-B-M complex to the membrane (*5*). These data initially suggested that Bcl10, Malt1, and CARD11 played a specific role in triggering antigen-receptor–dependent responses in lymphocytes. However, of the three proteins, only expression of CARD11 is restricted to cells of the lymphoid lineage. In contrast, Bcl10 and Malt1 are expressed in many different tissues (*2*). Data about mast cell activation provided the first clue that Bcl10-Malt1 function is not restricted to lymphocytes. Mast cells express Fcε receptors (FcεR), which are structurally related to T and B cell receptors. Cross-linking of FcεRI (the high-affinity receptor for the Fc portion of IgE)–bound IgE with a multivalent antigen triggers mast cell degranulation and production of inflammatory cytokines. FcεRI clustering or PKC activation of mast cells from mice lacking Bcl10 or Malt1 fails to induce production of the inflammatory cytokines TNFα (tumor necrosis factor α) and IL-6 (interleukin 6), which correlates with defective IKK and NF-κB signaling (*9*, *10*).

CARD10-Bcl10-Malt1 Signalosome Triggers GPCR Signaling to NF-κB

Two recent studies implicate Bcl10 and Malt1 in lysophosphatidic acid (LPA)–induced NF-κB activation in mouse embryonic fibroblasts (MEFs) (*1*, *3*). LPA is a potent bioactive lipid that binds to LPA receptors (1 to 4) and elicits effects on cell proliferation, survival, and migration. LPA receptors belong to the large family of GPCRs or seven-transmembrane receptors. GPCR engagement with structurally diverse ligands, including hormones, neurotransmitters, odorants, certain tastants, light, chemokines, and calcium, initiates the intracellular recruitment of heterotrimeric guanine nucleotide–binding proteins (G proteins) to the receptor. G proteins consist of three subunits, the Gα subunits that are responsible for guanosine triphosphate (GTP) and guanosine diphosphate (GDP) binding and GTP hydrolysis and the associated β and γ subunits (Gβ/γ). Ligand binding stimulates an exchange of GDP to GTP on Gα, which provokes dissociation of Gβ/γ from Gα, thereby activating the G protein. Certain types of GPCRs promote inflammatory reactions by modulating the activity of NF-κB, a process that involves signaling through members of the $G\alpha_{12/13}$, $G\alpha_q$, and $G\alpha_i$ families (*11*). PKC activity is required for mediating LPA-induced NF-κB activation, which suggests that similar mechanisms could be involved downstream of antigen receptors and GPCRs (*12*, *13*). Indeed, MEFs deficient in Bcl10 show strongly impaired NF-κB activation and reduced expression of the inflammatory cytokines IL-6 and macrophage inflammatory protein-2 (MIP-2) in response to LPA stimulation (*1*, *3*). By blocking $G\alpha_{12}$- and $G\alpha_q$-induced NF-κB activation with small interfering RNAs (siRNAs) directed against Bcl10, Lin and coworkers demonstrated that G proteins are involved in Bcl10-dependent NF-κB activation (*3*). In addition, Malt1 deficiency in MEFs abrogates NF-κB activation in response to LPA, which suggests that Bcl10-Malt1 complexes direct GPCR signaling to IKK and NF-κB (*1*).

By analyzing angiotensin II (Ang II)–mediated signal transduction, McAllister-Lucas *et al*. provided evidence that

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CARD10 (also known as CARMA3), which is structurally closely related to CARD11, bridges G-protein activation to the Bcl10-Malt1-IKK-NF-κB pathway (*2*). In addition to its effects on blood pressure, Ang II is known for its proinflammatory actions. Ang II binds to the type 1 Ang II receptor (AT_1R), a GPCR expressed in the liver. In hepatocytes, deficiency in CARD10 or Bcl10 and siRNA-mediated inactivation of Malt1 impaired Ang II induction of NF-κB (*2*). Even though it must be formerly proved for other GPCR agonists, the data indicate that the CARD10 (CARMA3)–Bcl10-Malt1 (C10-B-M) signalosome is a general regulator for GPCR signaling to the inflammatory NF-κB pathway. The mechanistic details of CARD10 activation and the specific PKC isoforms that link Gα to CARD10 remain to be identified; however, at least for LPA signaling, a prominent function of PKCδ in linking GPCR signaling to the CARD10-Bcl10-Malt1-IKK-NF-κB pathway can be assumed (*12*).

CARD9-Bcl10-Malt1 Signalosome Controls NF-κB in Response to Fungal Recognition

CARD9 structurally resembles CARD10 and CARD11 in that it contains an N-terminal CARD and an adjacent coiled-coil motif, but it lacks the C-terminal MAGUK domain (*14*). Analysis of CARD9-deficient mice strengthens the concept that different CARD scaffolds provide platforms for Bcl10-Malt1 complexes to trigger NF-κB activation. CARD9 is expressed in various tissues, including spleen, lung, and peripheral blood lymphocytes. It binds to Bcl10 and activates NF-κB, which suggests a role in antigen receptor signaling. However, Ruland and co-workers found no evidence that CARD9 is involved in TCR- and BCR-dependent signal transduction (*4*). Instead, CARD9 serves the functions of a typical CARD scaffold in bone-marrow–derived dendritic cells (BMDCs) by directing antifungal responses initiated by Dectin-1 to the NF-κB pathway. Dectin-1 belongs to the group of non–Toll-like receptor (TLR)–type pattern-recognition receptors (PRR) that recognize zymosan, a component of the yeast cell wall (*15*). Consequently, clearance of fungal *Candida albicans* cells was strongly impaired in $CARD9^{-/-}$ mice (*4*). Zymosan can also bind to TLR2, but using BMDCs deficient in the TLR adaptor Myd88, Gross *et al*. demonstrated that Dectin-1 is the critical zymosan receptor for the induction of CARD9-dependent NF-κB activation and cytokine production (*4*). NF-κB activation and induction of inflammatory cytokines by zymosan was also abolished in $Bcl10^{-/-}$ and $Malt1^{-/-}$ BMDCs, which suggests that a C9-B-M signalosome with crucial functions forms in innate immunity. Recently, a link between CARD9 and mitogen-activated protein kinase (MAPK) activation was reported for intracellular pathogen reception by NOD2 (nucleotide-binding oligomerization domain protein 2), but no effect on NF-κB signaling was observed (*16*). Thus, the C9-B-M signalosome may selectively channel fungal recognition to IKK and NF-κB signaling.

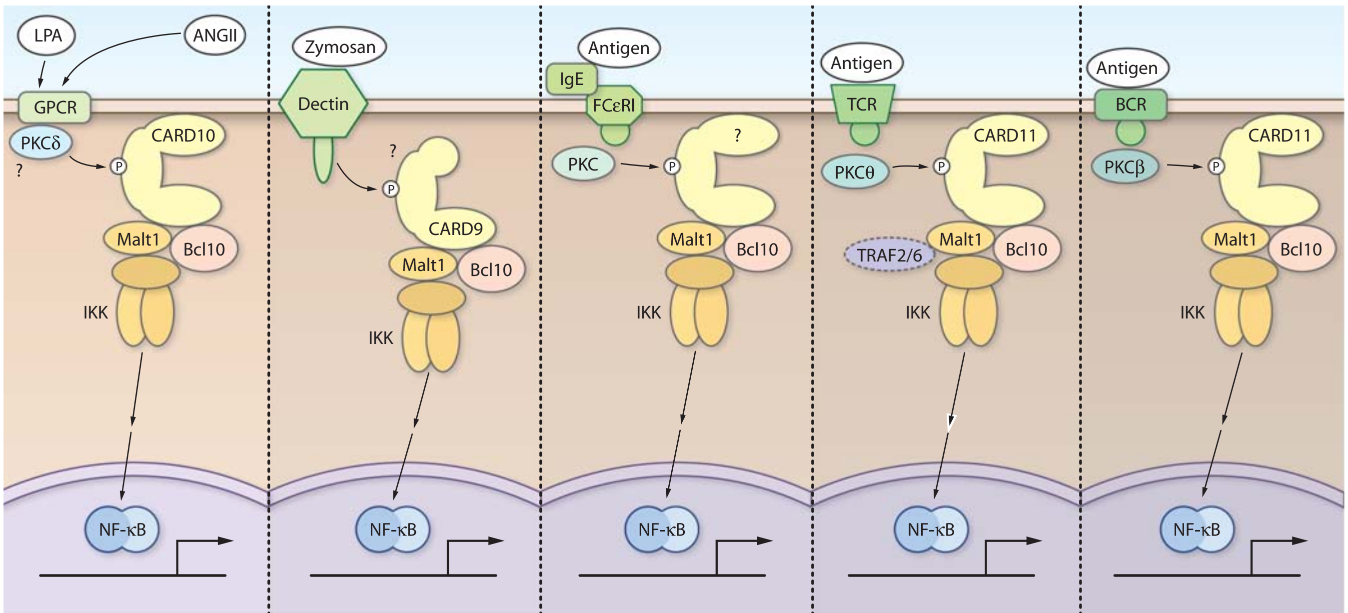

Fig. 1. Conserved Bcl10-Malt1 complexes interact with different CARD scaffolds to link various receptors to IKK and NF-κB signaling. TCR and BCR signaling in lymphocytes involves the PKCθ- or PKCβ-dependent CARD11, Bcl10, and Malt1 complex. In T cells, TRAF2 and TRAF6 couple the C11-B-M signalosome to the IKK complex. In response to FcεRI signaling in mast cells, Bcl10-Malt1 associates with an unknown CARD protein. In dendritic cells, antifungal recognition by Dectin-1 initiates C9-B-M–dependent NF-κB activation. GPCRs require the C10-B-M signalosome for activation of proinflammatory NF-κB.

Outlook

These studies establish an essential switch function of the C-B-M signalosome for activation of NF-κB signaling by showing that diverse receptors can connect to the conserved Bcl10-Malt1 complex through distinct CARD coiled-coil scaffolds. Thus, evolutionarily conserved mechanisms connect fungal recognition (Dectin-1), antigen binding (TCR, BCR, and FcεRI), and GPCRs (LPA and AT_1R) to IKK and NF-κB. Because no function for placental CARD14 (also known as CARMA2) has been assigned, it is likely that more receptors will be identified that link to NF-κB through C-B-M complexes (*17*). Further, the ability of the distantly related CARD9 protein to participate in a C-B-M signalosome hampers our ability to predict functional homologs (*14*). The results indicate that binding of Bcl10 is the minimal requirement for a CARD scaffold to connect to NF-κB through the Bcl10-Malt1 module. A systematic analysis of the ability of Bcl10 to associate with other CARD-containing proteins might lead to the identification of further processes that trigger C-B-M–dependent NF-κB signaling. Note that, with the exception of Bcl10, which plays a role in neural tube closure in the developing embryo (*18*), C-B-M components seem to be largely dispensable for vital developmental or differentiation processes.

Almost all mechanistic insights into CARD-Bcl10-Malt1 regulation arose from research in T and B cells. A considerable amount of biochemical work is needed to elucidate and to compare the activation of the different C-B-M signalosomes downstream of TCRs and BCRs, FcεRI, GPCRs, and Dectin-1. Analysis of CARD9 function will be particularly informative, because it lacks the MAGUK domain; it will be interesting to see whether CARD9 associates with the plasma membrane and is targeted by PKCs. One important issue concerns the mechanism of C-B-M signalosome–mediated IKK activation. In T cells, Bcl10-Malt1 complexes induce a regulatory ubiquitination of IKKγ that seems to be catalyzed by TRAF2 (tumor-necrosis-factor-receptor–associated factor 2) and TRAF6 (*19*, *20*). Ang II stimulates IKKγ ubiquitination, which suggests that similar events take place downstream of the GPCR pathways, but an involvement of TRAFs in GPCR signaling remains to be proven (*2*). Negative regulatory events that involve phosphorylation and degradation of Bcl10 impinge on the C11-B-M complex in lymphocytes (*21–24*); Klemm *et al*. have observed PKC-dependent Bcl10 degradation following LPA stimulation of MEFs, indicating that conserved mechanisms for postinductive inactivation exist (*1*). Finally, constitutive activation of antiapoptotic NF-κB signaling contributes to tumor formation. Inactivation of CARD11, Bcl10, or Malt1 impairs the survival of a subset of cells derived from malignant lymphomas (*25*). The functional identification of C-B-M signalosomes outside the immune system suggests that survival of other tumors cells may also depend CARD-Bcl10-Malt1 signaling. The disruption of C-B-M signalosomes may thus represent an attractive therapeutic strategy in certain types of tumors.

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