MALT1/Paracaspase Is a Signaling Component Downstream of CARMA1 and Mediates T Cell Receptor-induced NF-κB Activation*

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T cell receptor (TCR) induces a series of signaling cascades and leads to activation of multiple transcription factors, including NF-κB. Although the mechanism of TCR-induced NF-κB activation is not fully understood, recent studies indicate that Bcl10 and CARMA1, two adaptor/scaffold proteins, play essential roles in mediating TCR-induced NF-κB activation. MALT1/paracaspase is a caspase-like protein that contains an N-terminal death domain, two Ig-like domains, and a C-terminal caspase-like domain. It binds to Bcl10 through its Ig-like domains and cooperates with Bcl10 to activate NF-κB. Recently, it has been shown that MALT1 is involved in mediating TCR signal transduction, leading to activation of NF-κB. In this study, we show that MALT1 is recruited into the lipid rafts of the immunological synapse following activation of the TCR and the CD28 coreceptor (CD3/CD28 costimulation). This recruitment of MALT1 is dependent on CARMA1 because CD3/CD28 costimulation failed to recruit MALT1 into lipid rafts in CARMA1-deficient T cells. In addition, we also found that MALT1 not only binds to Bcl10 directly, but also associates with CARMA1 in a Bcl10-independent manner. Therefore, MALT1, Bcl10, and CARMA1 form a trimolecular complex. Expression of a MALT1 deletion mutant containing only the N-terminal death domain and the two Ig-like domains completely blocked CD3/CD28 costimulation-induced, but not tumor necrosis factor-α-induced, NF-κB activation. Together, these results indicate that MALT1 is a crucial signaling component in the TCR signaling pathway.

T cell activation plays a critical role in regulation of the immune response. Dysregulation of this process results in cancer, autoimmunity, and immunodeficiency diseases. T cell activation is initiated by major histocompatibility complex molecules on antigen-presenting cells presenting antigen peptides to T cell receptors (TCRs)1 on the surface of T cells. This stimulation of TCR-CD3 complexes induces a series of signal transduction events leading to activation of multiple transcription factors such as AP-1, NF-AT, and NF-κB (1–3). However, stimulation of TCR/CD3 complexes alone is insufficient to activate T cells. Instead, this signal will induce T cell anergy or apoptosis. The complete activation of T cells requires a second “costimulatory” signal generated through interactions between B7-1(CD80)/B7-2(CD86) molecules on antigen-presenting cells and CD28 receptors on T cells. This combined stimulation of TCR/CD3 and CD28 molecules (known as CD3/CD28 costimulation) leads to optimal activation of multiple transcription factors, including NF-κB, and subsequent production of interleukin-2 and other cytokines (4).

The NF-κB family of transcription factors plays a critical role in controlling expression of various cytokine and anti-apoptotic genes. NF-κB activity is regulated through interactions with a series of cytoplasmic inhibitory proteins termed IκB, which mask the nuclear localization signal of NF-κB, thereby sequestering NF-κB in the cytoplasm. Treatment of cells with various stimuli such as tumor necrosis factor-α (TNF-α), interleukin-1β, and CD3/CD28 costimulation initiates signal transduction cascades leading to activation of IκB kinase (IKK). IKK phosphorylates two regulatory serines located in the N terminus of IκB, triggering rapid ubiquitination and proteolysis of IκB in the 26 S proteasome complex (5). Degradation of IκB molecules unMASKS the nuclear localization sequence of NF-κB. NF-κB that is released from IκBα rapidly translocates into the nucleus, where it regulates the transcription of various genes centrally involved in immune and anti-apoptotic responses (6).

Following CD3/CD28 costimulation, a large multicomponent complex at the contact area between the T cell and the antigen-presenting cell is formed, termed the supramolecular activation complex or the immunological synapse (7). The contact area of the T cell is highly enriched in cholesterol and glycosphingolipids, also termed lipid rafts. Some signaling molecules such as Lck and LAT are constitutively associated with the lipid rafts, whereas other key signaling molecules such as protein kinase Cθ (PKCθ), ZAP70, Vav, SLP-76, and IKKβ are recruited into the lipid rafts following CD3/CD28 costimulation (8). Although it is not fully understood how signaling induced by CD3/CD28 costimulation leads to activation of NF-κB, recent studies indicate that phospholipase Cγ1 is essential for CD3/CD28 costimulation-induced NF-κB activation (9). The activated phospholipase Cγ1 hydrolyzes inositol phospholipids into inositol mono- and diacylglycerols. The production of inositol polyphosphates then leads to an elevation of calcium through mobilization of intracellular stores. Subsequently, the elevated intracellular calcium and diacylglycerol activate PKC. Among the multiple isoforms of PKC that are activated following CD3/CD28 costimulation, only PKCθ, a Ca2+-independent PKC isoform, is recruited into membrane lipid rafts of the immunological synapse. Previous studies in-

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‡ The abbreviations used are: TCR, T cell receptor; TNF-α, tumor necrosis factor-α; IKK, IκB kinase; PKCθ, protein kinase Cθ; PMA, phorbol 12-myristate 13-acetate; MALT, mucosa-associated lymphoid tissue; HEK293, human embryonic kidney 293; MES, 4-morpholineethanesulfonic acid; ERK, extracellular signal-regulated kinase; JNK, c-Jun-N-terminal kinase.

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indicate that PKCδ/H9258 plays an essential role in activation of IKK and NF-kB induced by CD3/CD28 costimulation or phorbol 12-myristate 13-acetate (PMA), a pharmacological homolog of diacylglycerol, in mature T cells (10–12). However, the molecular mechanism by which PKCδ/H9258 activates IKK remains to be determined.

CARMA1 (also known as CARD11) is a scaffold molecule containing an N-terminal caspase recruitment domain, a coiled-coil domain, a PDZ domain, an SH3 (Ssrc homology-3) domain, and a C-terminal GUK (guanylate kinase-like) domain (13). PDZ-SH3-GUK is a signature motif of MAGUK (membrane-associated guanylate kinase) proteins that play important roles in regulation of the interface between membrane components and cytoskeletal proteins (14). Consistent with the functions of MAGUK family proteins, CARMA1 is constitutively associated with the cytoplasmic membrane and is recruited into the immunological synapse following CD3/CD28 costimulation (15). Recent studies also indicate that CARMA1 links PKCδ to downstream signaling components leading to activation of NF-κB (15–17). Gene targeting experiments further confirm that CARMA1 is an essential molecule for antigen receptor-induced NF-κB activation (18–21). More recently, our studies suggested that PKCδ/H9258 is recruited into the immunological synapse through an interaction with CARMA1 (22).

Following CD3/CD28 costimulation, CARMA1 also recruits Bcl10, an adaptor protein, into the immunological synapse (15). Bcl10 is a 233-residue protein that contains an N-terminal caspase recruitment domain and a C-terminal Ser/Thr-rich domain (23–28). Genetic targeting experiments demonstrated that Bcl10 is an essential signaling component that lies downstream of PKCδ/H9258, but upstream of IKK in the TCR signaling pathway (29). Besides its interaction with CARMA1, Bcl10 also

**Fig. 1.** MALT1 is recruited into lipid rafts following CD3/CD28 costimulation. Jurkat cells (~3–5 × 10⁶) were stimulated with or without anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) antibodies. The cells were then lysed with 1% Triton X-100 and subjected to sucrose density gradient centrifugation to isolate lipid rafts. A, proteins from equal volumes of representative collected fractions were separated by SDS-PAGE and analyzed by Western blotting (WB) using antibodies against Bcl10 or Lck. B, the lipid raft (A, lanes 1–5) or cytosol (A, lanes 8 and 9) fractions were pooled together. The pooled samples were immunoprecipitated (IP) using monoclonal antibodies against the C terminus of MALT1. The immunoprecipitates (upper panel) and lysates (lower panel) were subjected to SDS-PAGE and analyzed by Western blotting using anti-antibodies against the N terminus of MALT1 or Lck, respectively.

**Fig. 2.** MALT1 is a signaling component downstream of CARMA1. A, Jurkat or JPM50.6 cells (~3–5 × 10⁶) were stimulated with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) antibodies for various time points. The lipid raft fractions were prepared as described in the legend to Fig. 1. The pooled fractions of the lipid rafts or cytosol were immunoprecipitated (IP) with antibodies against the C terminus of MALT1 and then subjected to SDS-PAGE and analyzed by Western blotting (WB) using antibodies against the N terminus of MALT1. A portion of the pooled fractions were directly subjected to SDS-PAGE and Western blotting using antibodies against various signaling components. B, Jurkat T or JPM50.6 cells were transfected in triplicate with expression plasmids encoding an NF-κB-dependent luciferase reporter gene in the presence or absence of expression plasmids encoding PKCδ/AE or cIAP-MALT1. Twenty-four hours after transfection, the transfected cells were lysed, and luciferase activity was determined. All of the samples were also transfected with an elongation factor-1α promoter Renilla luciferase. The constitutively expressed Renilla luciferase activity was used to normalize the transfection efficiency. All data are presented as fold induction with S.D. values shown.
binds to MALT1 (mucosa-associated lymphoid tissue protein-1, also known as paracaspase) (30, 31). MALT1 contains an N-terminal death domain, followed by two Ig-like domains and a C-terminal caspase-like domain. Chromosomal translocation of the MALT1 gene to the genomic locus of the cIAP gene has been linked to mucosa-associated lymphoid tissue (MALT) lymphomas (30, 31). Importantly, overexpression of cIAP-MALT1 fusion proteins derived from MALT lymphoma patients potently activates NF-κB, suggesting that MALT1 may be involved in regulation of IKK activation. Therefore, it has been proposed that Bcl10 may induce oligomerization of MALT1 through binding to the Ig-like domains of MALT1, leading to activation of the IKK complex (30, 31). More recently, gene targeting experiments indicated that MALT1 is required for TCR-induced NF-κB activation (32, 33), however, it remains to be determined how MALT1 is involved in TCR-induced NF-κB activation.

In this study, we found that MALT1 is recruited into the lipid rafts of the immunological synapse in a CARMA1-dependent manner and forms a complex with Bcl10 and CARMA1 following CD3/CD28 costimulation. Stable expression of a deletion mutant MALT1 completely abolished CD3/CD28 costimulation-induced NF-κB activation. Together, these results indicate that MALT1 is a crucial signaling component involved in the TCR signal transduction pathway.

EXPERIMENTAL PROCEDURES

Stable Transfected Cell Lines and Cell Cultures—Jurkat T and JPM50.6 cells (16) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum at 37 °C and 5% CO2. HEK293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 0.5% CO2 and were selected in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 0.5 μg/ml G418. Human embryonic kidney 293 (HEK293) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 0.5 μg/ml G418. Yeast two-hybrid interaction assays were performed in yeast strain AH109 and transformed by a standard lithium acetate method.

Plasmids and Antibodies—Plasmids encoding FLAG-tagged MALT1 and its mutants were generated by PCR using the original cDNAs (30) as templates and subcloned into the pcDNA3.1(-) vector in-frame with a C-terminal FLAG epitope tag. Plasmids encoding CARMA1, PKCθ, and Bcl10 were described previously (16). Plasmids for the yeast two-hybrid interaction assay were generated by inserting sequence encoding CARMA1 deletion mutants downstream of the Gal4 DNA-binding domain in the pGBK-T7 vector (Clontech) and subcloning the caspase-like domain (residues 333–824) of MALT1 downstream of the Gal4 activation domain in the pGAD-T7 vector (Clontech). Monoclonal antibodies against both the N and C termini of MALT1 were generated in the Genentech central production facility. Antibodies against PKCθ (clone 27) and LAT were purchased from BD Transduction Laboratories (San Diego, CA), and antibodies against Bcl10 (H197), Lck, and the FLAG epitope tag were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Lipid Raft Purification—Detergent-insoluble fractions were separated as described (34). Briefly, Jurkat or JPM50.6 T cells (~3–5 × 106) were lysed in 1 ml of MNE buffer (25 mM MES (pH 6.5), 150 mM NaCl, 5 mM EDTA, 30 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10% glycerol).
date, and 10 μg/ml each protease inhibitor) with 1% Triton X-100 for 20 min on ice and homogenized 15 times in a Dounce homogenizer. Samples were centrifuged at 1000 × g for 10 min at 4 °C. The supernatants were mixed with 1 ml of 80% sucrose in MNE buffer and transferred to a Beckman ultracentrifuge tube. Next, 2 ml of 30% sucrose followed by 1 ml of 5% sucrose in MNE buffer were overlaid. Samples were centrifuged in an STi-55 rotor at 200,000 × g for 20 h. Fractions (400 μl/fraction) were collected from the top of the gradient. Proteins from each fraction were precipitated with trichloroacetic acid before separation by 10% SDS-PAGE.

Co-immunoprecipitation—Myc-tagged CARMA1 was transfected into HEK293 cells expressing different versions of MALT1 by calcium phosphate precipitation. Twenty hours later, MALT1 proteins were immunoprecipitated with anti-FLAG antibody M2 affinity gel (Sigma). Samples were washed four times with lysis buffer (50 mM HEPES (pH 7.4), 1 mM EDTA, 150 mM NaCl, and 1% Nonidet P-40) and eluted with FLAG peptide (Sigma). The eluted samples were subjected to SDS-PAGE and Western blotting.

RESULTS

To determine whether MALT1 is involved in TCR signal transduction, we examined whether CD3/CD28 costimulation induces MALT1 translocation to lipid rafts. The detergent-insoluble membrane (lipid raft) fractions of Jurkat T cells were prepared by centrifugation of cellular components in a discontinuous sucrose gradient. The recruitment of signaling components (Bcl10 and Lck) into lipid rafts was examined by Western blotting of individual fractions of the sucrose gradient (Fig. 1A). To determine whether MALT1 was present in the lipid raft fractions, we combined the sucrose gradient fractions that contained lipid rafts (Fig. 1A, lanes 1–5) and then immunoprecipitated MALT1 from the pooled fractions. We found that a small portion of MALT1 was present in the lipid raft fractions in Jurkat T cells following CD3/CD28 costimulation (Fig. 1B), suggesting that MALT1 may be a signaling component in the TCR signal transduction pathway.

To determine the molecular mechanism of CD3/CD28 costimulation-induced recruitment of MALT1 into lipid rafts, we stimulated Jurkat T cells or CARMA1-deficient Jurkat T cells (JPM50.6) (16) with anti-CD3 and anti-CD28 antibodies for different time points. The recruitment of MALT1 and other components in these cells was also detected by Western blotting. CD3/CD28 costimulation rapidly recruited MALT1 into lipid rafts, and this recruitment was diminished within 20 min (Fig. 2A). The recruitment of MALT1 into the lipid rafts appears to be dependent on CARMA1 because CD3/CD28 costimulation failed to induce the recruitment of MALT1 into lipid rafts in JPM50.6 cells (Fig. 2A). Similarly, Bcl10 and PKCδ translocation to lipid rafts induced by CD3/CD28 costimulation was defective in JPM50.6 cells (Fig. 2A), as described previously (22).

Chromosomal translocation of the MALT1 gene to the genomic locus of the cIAP gene was found to cause expression of a cIAP-MALT1 fusion protein in a MALT lymphoma patient. Expression of this cIAP-MALT1 fusion protein (also known as
paracaspase-Case2) potently activates NF-κB (30, 31). To determine whether MALT1 functions downstream of CARMA1, we expressed cIAP-MALT1 in JPM50.6 cells to test whether cIAP-MALT1-induced NF-κB activation is dependent on CARMA1. Consistent with the above results, expression of cIAP-MALT1 effectively activated NF-κB in JPM50.6 cells (Fig. 2D), whereas PKC⁰(AE), a constitutively active version of PKC⁰ that functions upstream of CARMA1 (16), failed to do so (Fig. 2B). Together, these results indicate that MALT1 is a signaling component downstream of CARMA1 in the TCR signal transduction pathway.

It has been shown that MALT1 associates with Bcl10 through its N-terminal domain (31). To investigate the role of MALT1 in the TCR signal transduction pathway, we stably transfected constructs encoding various MALT1 mutants into Jurkat T cells. These included a mutant consisting of N-terminal residues 1–332 (MALT1(N-Term)), which contain the N-terminal death domain and the Ig-like domains, and a mutant consisting of C-terminal residues 333–824 (MALT1(C-Term)), which contain the caspase-like domain (Fig. 3A). Activation of NF-κB in these cells was examined following various stimuli. Although the expression levels of transfected MALT1(N-Term) and endogenous MALT1 were comparable in the cells stably expressing MALT1(N-Term) (Fig. 3B, lanes 1–6), both CD3/CD28 costimulation and PMA/CD28 costimulation failed to induce NF-κB activation in the cells expressing MALT1(N-Term) (Fig. 3C), whereas TNF-α effectively induced NF-κB activation. The inhibitory effect caused by expression of MALT1(N-Term) is specific to the NF-κB pathway because CD3/CD28 costimulation effectively induced AP-1 activation in these cells (Fig. 3D). In contrast, expression of MALT1(C-Term), whose level was comparable with the expression level of MALT1(N-Term) (Fig. 3B, lanes 7–10), had no inhibitory effects on NF-κB activation (Fig. 3C). Instead, expression of MALT1(C-Term) appeared to enhance NF-κB activation. This finding suggests that MALT1 is specifically involved in TCR-induced NF-κB activation.

To determine which level of the TCR signaling cascade is blocked in Jurkat T cells expressing MALT1(N-Term), we examined CD3/CD28 costimulation-induced IKK activation. We found that CD3/CD28 or PMA/CD28 costimulation, but not TNF-α, failed to activate IKK in the MALT1(N-Term) cells (Fig. 4A), whereas expression of MALT1(C-Term) in Jurkat T cells had no inhibitory effects on CD3/CD28 or PMA/CD28 costimulation.

Fig. 5. CARMA1-induced NF-κB activation is partly blocked in T cells expressing MALT1(N-Term). Jurkat T cells or Jurkat T cells expressing MALT1(N-Term) were transfected in triplicate with plasmids encoding an NF-κB-dependent reporter gene in the presence or absence of expression plasmids encoding CARMA1. Twenty-four hours after transfection, the transfected cells were lysed, and luciferase activity was determined. All of the samples were also transfected with elongation factor-1α-luciferase activity was used to normalize the transfection efficiency. All data are presented as fold induction with S.D. values shown.

Fig. 6. Endogenous MALT1 is effectively recruited into lipid rafts in Jurkat T cells expressing MALT1(N-Term) following CD3/CD28 costimulation. Jurkat T cells or Jurkat T cells expressing MALT1(N-Term) (5–3 × 10⁶) were stimulated with or without anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) antibodies. Lipid rafts from these cells were prepared using the same procedure as described in the legend to Fig. 1. The lipid raft fractions were pooled together. The pooled samples were immunoprecipitated (IP) using antibodies against the C terminus of MALT1. The immunoprecipitates (upper panel) and the pooled lipid raft samples (lower panel) were subjected to SDS-PAGE and analyzed by Western blotting using anti-antibodies against the N terminus of MALT1 or Lck, respectively. IB, immunoblot.

Fig. 7. MALT1 is associated with CARMA1 in a Bcl10-independent manner. HER293 cells or HEK293 cells stably expressing MALT1, MALT1(N-Term), or MALT1(C-Term) were transiently transfected with or without Myctagged CARMA1. Twenty-four hours after transfection, the transfected cells were lysed. The total cell lysates were either immunoprecipitated using anti-FLAG antibody-conjugated agarose (A) or directly subjected to Western blotting (WB) using anti-Myc or anti-Bcl10 antibodies (B). The immunoprecipitates were eluted with FLAG epitope peptides and subjected to Western blotting using anti-Bcl10, anti-Myc, or anti-FLAG antibodies.
lation-induced IKK activation (Fig. 4A). Furthermore, activation of mitogen-activated protein kinases ERK and JNK induced by CD3/CD28 costimulation was intact in the MALT1(N-Term) cells (Fig. 4, B and C). Together, these results indicate that expression of MALT1(N-Term) specifically inhibits a signaling event upstream of the IKK complex in the TCR signaling pathway.

To further demonstrate that MALT1 is a downstream component of CARMA1, we determined CARMA1-induced NF-κB activation by overexpressing CARMA1 either in Jurkat T cells or in the cells expressing MALT1(N-Term). Expression of CARMA1 effectively activated NF-κB in Jurkat T cells, whereas activation of NF-κB was partially blocked in the cells expressing MALT1(N-Term) (Fig. 5). This result is consistent with the above data (Fig. 2), indicating that MALT1 is a downstream component of CARMA1 in the TCR signaling pathway.

To determine whether expression of MALT1(N-Term) in Jurkat T cells affects the recruitment of endogenous MALT1 into the lipid rafts following CD3/CD28 costimulation, the lipid raft fractions were prepared from Jurkat T cells or the cells expressing MALT1(N-Term) with or without CD3/CD28 costimulation. The endogenous MALT1 proteins were immunoprecipitated using antibodies against the C terminus of MALT1. We found that the endogenous MALT1 proteins were effectively recruited into the lipid rafts in Jurkat T cells as well as in the cells expressing MALT1(N-Term) following CD3/CD28 costimulation (Fig. 6).

Previous studies indicate that CARMA1 associates with Bcl10. Since the N-terminal domain of MALT1 was shown to associate with Bcl10 (31), we examined the molecular basis by which MALT1 associates with the Bcl10-CARMA1 complex. We stably transfected HEK293 cells with plasmids encoding FLAG-tagged MALT1, MALT1(N-Term), or MALT1(C-Term). These cells were then transiently transfected with or without Myc-tagged CARMA1 (Fig. 7B). The complex formation of transfected MALT1 and CARMA1 with Bcl10 was examined by co-immunoprecipitation experiments. Specifically, FLAG-tagged MALT1 or its deletion mutants were first immunoprecipitated using anti-FLAG antibody-conjugated agarose beads. The proteins associated with the immunoprecipitation complexes were then detected by immunoblot analysis. Consistent with previous studies, both MALT1 and MALT1(C-Term) associated with CARMA1 (Fig. 7A, upper panel, lanes 4 and 8), and this interaction is apparently independent of Bcl10 because MALT1(C-Term) could not associate with Bcl10 (middle panel, lanes 7 and 8), suggesting that the caspase-like domain of MALT1 may directly associate with CARMA1.

To further demonstrate that the caspase-like domain of MALT1 can directly interact with CARMA1, we use the yeast two-hybrid interaction system to confirm this interaction. We found that the C-terminal caspase-like domain (residues 333–824) of MALT1 is specifically associated with the coiled-coil domain of CARMA1 in the yeast two-hybrid interaction system (Fig. 8). The coiled-coil domain of CARMA1 has been shown to be required for TCR-induced NF-κB activation (17). The association of the coiled-coil domain of CARMA1 with MALT1 may...

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**Yeast Two-hybrid Interaction Assay**

| CARMA1 and its deletion mutants | Interaction with MALT1(C-Term) |
|----------------------------------|-------------------------------|
| CARMA1                           | ![Diagram](https://example.com/diagram.jpg) |
| CARMA1(CD-CC)                    | +                             |
| CARMA1(CD)                       | -                             |
| CARMA1(CC)                       | +                             |
| CARMA1(MAGUK)                    | -                             |

**Fig. 8.** The caspase-like domain of MALT1 is directly associated with the coiled-coil domain of CARMA1.

**Fig. 9.** Working model of TCR-induced NF-κB activation through the CARMA1-Bcl10-MALT1 complex. MHC, major histocompatibility complex; APC, antigen-presenting cell; C-C, coiled-coil domain; CARD, caspase recruitment domain; S/T, Ser/Thr-rich domain; DD, death domain.
provide the molecular mechanism by which CARMA1 regulates its downstream signaling components. Together, these results indicate that MALT1 forms a trimolecular complex with Bcl10 and CARMA1 in the TCR signal transduction pathway (Fig. 9).

**DISCUSSION**

MALT1/paracaspase is a protein that contains an N-terminal death domain, two Ig-like domains, and a C-terminal caspase-like domain. A fusion protein of cIAP-MALT1 resulting from a chromosomal translocation of the MALT1 gene to the genomic locus of the cIAP gene is associated with MALT lymphoma. Overexpression of the oncopgenic cIAP-MALT1 fusion protein potently activated NF-κB, suggesting that cIAP-MALT1 may constitutively induce NF-κB activation and contribute to the malignancy of MALT lymphoma. However, the functions of MALT1 in normal cellular signaling pathways have not been defined. Previous studies show that MALT1 is physically associated with Bcl10, suggesting that MALT1 is involved in the Bcl10-dependent signaling pathway such as antigen receptor signaling in lymphocytes. In this study, we demonstrate that MALT1 is translocated to the lipid raft fractions following CD3/CD28 costimulation. This result indicates that MALT1 is a signaling component in the TCR signaling pathway. In agreement with recent gene targeting experiments, we found that the stable deletion of a deletion mutant of MALT1 in Jurkat T cells completely blocked CD3/CD28 costimulation-induced NF-κB activation, indicating that MALT1 indeed plays a critical role in mediating TCR-induced NF-κB activation.

Emerging evidence indicates that multiple signaling components are recruited into lipid rafts following stimulation of the TCR complex. Our data indicate that a portion of MALT1 is also recruited into lipid rafts. This recruitment is apparently dependent on CARMA1, an essential scaffold molecule for TCR-induced NF-κB activation. This result is consistent with the previously proposed model that MALT1 is downstream of CARMA1 in the TCR signaling pathway. However, we also found that MALT1 forms a complex with CARMA1 in a Bcl10-independent manner. This finding is different from the proposed model, in which CARMA1-Bcl10-MALT1 is in a linear arrangement in a signaling pathway. Our results suggest a working model that CARMA1, MALT1, and Bcl10 may form a trimolecular complex following CD3/CD28 costimulation (Fig. 9). Therefore, CARMA1 may directly recruit MALT1 into lipid rafts, where CARMA1 either induces MALT1 activity or bridges MALT1 and its substrates. In this model, Bcl10 and MALT1 may cooperatively regulate the downstream IKK activity.

Although MALT1 contains a caspase-like domain, it remains to be determined whether MALT1 functions as a caspase-like enzyme or has other enzymatic activities. Of note, a mutant version of MALT1 in which a conserved Cys residue that is critical for caspase activity is mutated to Ala exhibited no inhibitory effects on CD3/CD28 costimulation-induced NF-κB activation (data not shown), suggesting that caspase-like activity may not be important for MALT1 function. Therefore, revealing the biological function of MALT1 will provide the mechanism by which MALT1 activates the downstream IKK complex.

In summary, we have demonstrated that MALT1 is an important signaling component downstream of CARMA1 and is recruited into lipid rafts of the immunological synapse following CD3/CD28 costimulation. MALT1 forms a trimolecular complex with CARMA1 and Bcl10 to mediate TCR-induced IKK activation, leading to activation of NF-κB. These observations are consistent with previous studies showing that Bcl10 and CARMA1 are specifically involved in TCR-induced NF-κB activation. Therefore, the next exciting task is to determine how MALT1 induces IKK activation.
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