Negative Regulation of T Cell Antigen Receptor-mediated Crk-L-C3G Signaling and Cell Adhesion by Cbl-b*

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It was previously reported that Cbl-b associates with Crk-L in Jurkat T cells. However, the physiological significance of such association remains unclear. Here we examined a regulatory role of Cbl-b in Crk-L-C3G signaling pathway. We found that Cbl-b associates with, and induces, ubiquitin conjugation to Crk-L, which requires a functional RING finger. Cbl-b deficiency does not affect Crk-L stability, but its association with C3G. In Crk-L-deficient T cells, the interaction between Crk-L and C3G, and the activity of the small GTPase Rap1, are increased. Cbl-b deficiency also increases phosphorylation and cell surface binding to ICAM-1, a finding that is supported by the enhanced clustering of LFA-1 in Cbl-b deficient T cells in response to TCR stimulation. Thus, Cbl-b plays a negative role in Crk-L-C3G-mediated Rap1 and LFA-1 activation in T cells.

Engagement of the T cell antigen receptor (TCR) by the antigenic peptide plus major histocompatibility complex (MHC) in the antigen presenting cells triggers rapid tyrosine phosphorylation and activation of protein-tyrosine kinases, the Src family (Lck and Fyn), and the Syk family (Zap-70). Activation of these kinases in turn induces tyrosine phosphorylation of a number of intracellular substrates including adaptor proteins to form intermolecular network (1, 2). Cbl is one of the adaptor proteins, which consists of an amino-terminal SH2-like domain, a RING finger, and carboxy-terminal proline-rich sequences with potential tyrosine phosphorylation sites (3). Previous studies from numerous laboratories have demonstrated that Cbl associates with a number of critical signaling molecules upon T cell activation including Zap-70, Grb-2, 14-3-3, phosphatidylinositol 3-kinase (PI3K), and Crk-L (3). In addition to its adaptor's role, Cbl also functions as an E3 ubiquitin (Ub) ligase, whose RING finger domain binds to a Ub-loaded protein. Cbl thus functions in negative regulation of Ras signal pathway in T cells (3). Whether Cbl-b is also involved in Rap1 activation in T cells remains to be determined. In this study, we examined whether Cbl-b acts as an E3 ligase for Crk-L and whether Cbl-b deficiency affects Crk-L-C3G signaling in response to TCR stimulation. The study may shed light on the biological function of Cbl-b in T cell activation and tolerance induction.

EXPERIMENTAL PROCEDURES

Antibodies and Plasmids—Polyonal antibodies specific to Cbl-b, Cbl-L, C3G, Fyn, Lck, and Grb2, and monoclonal antibodies (mAbs) specific to c-Myc, HA, Zap-70, Lck, Crk-L, and Ub were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). mAbs specific to Rap1, and Raf-1, were purchased from PharMingen (La Jolla, CA). Phosphotyrosine-specific mAbs was purified from culture supernatant of 4G10 B cell hybridoma, and mAb, anti-CD3e, was purified from the culture supernatant of B cell hybridoma 145-2C11. Cbl-b cDNA encoding wild-type Cbl-b, or Cbl-b RING finger mutants harboring Cys to Ala (Cbl-b CA), or Trp to Ala (Cbl-b WA) mutation, the Ub cDNA with a Myc epitope tag, and the Crk-L plasmid were described previously (9, 11). The bacterial expression plasmid containing glutathione S-transferase (GST)-RalGDS Rap1 binding domain (RBD) was provided by J. Bos. Preparation of GST fusion protein was performed as described previously (4).

Mice and T Cell Activation—Cbl-b deficient (Cbl-b−−) mice on a C57BL/6 background were originally provided by M. Naramura and H. Gu at the National Institutes of Health. Primary T cells were collected from lymph nodes of 6–8-week-old mice. Single-cell suspensions of T cells were prepared at 3 × 10⁶ cells/ml in RPMI 1640 (Irvine Scientific, Irvine, CA) supplemented with 5% fetal calf serum. T cells were either left untreated or were added with 10 μg/ml anti-CD3e. The cells were
incubated on ice for 10 min before stimulation at 37 °C for various times as indicated.

Cell Culture and Transfection—Jurkat-TAG T cells were grown in RPMI 1640 medium (Irvine Scientific) supplemented with 10% fetal bovine serum and antibiotics. For protein expression in Jurkat T cells, cells were transfected with an appropriate amount of plasmid (usually 1–5 µg in total) by electroporation (240 V, 850 microfarads; Bio-Rad). After 48 h, cells were harvested and cross-linked by incubating with 50 µg/ml of P-40, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 5 mM sodium pyrophosphate, 2 mM orthovanadate, and 10 µg/ml each aprotinin and leupeptin) for 30 min at 4 °C, and insoluble materials were removed by centrifugation at 15,000 × g at 4 °C for 20 min. For displaying ubiquitinated protein, 0.1% SDS and 5 µM mercaptoethanol were added into the lysis buffer to disrupt non-specific protein interactions.

Adhesion Assays—96-Well Nunc Maxisorp Immuno flat-bottomed plates were coated overnight at 4 °C with 10 µg/ml soluble recombinant murine ICAM-1-Fc (R&D Systems, Minneapolis, MN), washed, blocked with 2% BSA/PBS for 1 h at 37 °C, and then washed with medium. 1 × 10⁶ purified lymph node T cells in 100 µl of 2% BSA/PBS were mixed with 10 µg/ml of anti-CD3e or 20 µg/ml of phosphor 12-myristate 13-acetate. Cells were allowed to attach for 30 min at 37 °C and nonadherent cells removed with warm RPMI1640 medium. Adherent cells were quantified, and all data are expressed as the mean of the percentage of binding cells relative to total cell input from three replicates.

Flow Cytometric Analysis—Lymphocytes from Cbl-b−/− and Cbl−/− mice were stained with fluorescein isothiocyanate (FITC)-, phycoerythrin-, or cychrome C-conjugated antibodies to murine CD4, CD8, and CD11a (mAb, 2D10, PharMingen). The cells were preincubated with anti-FC-R for 10 min and stained with the antibodies on ice for 30 min. After a final wash, cells were analyzed on a FACScan flow cytometer (BD Biosciences), and the data were analyzed by using CellQuest software.

Measurement of Soluble ICAM-1 Binding—1 × 10⁶ lymphocytes were treated with or without 10 µg/ml anti-CD3 antibody or 10 µg/ml phorbol 12-myristate 13-acetate for 30 min at 37 °C. Cells were then washed in PBS containing 0.1% BSA, incubated with 10 µg/ml soluble recombinant ICAM-1-Fc (R&D Systems) 30 min at 37 °C, washed twice, and then incubated with 10 µg/ml Fe-specific FITC-conjugated rabbit anti-human IgG (Jackson Immunoresearch, West Grove, PA) for 1 h at 4 °C. Unbound secondary antibody was removed by washing twice and fluorescence of live cells detected using the FACScan flow cytometer.

Rap1 Activation Assay—T lymphocytes were incubated in cold RPMI 1640 with or without anti-CD3 antibody for 30 min on ice. Cells were then washed twice with cold medium and activated for the indicated time periods with 20 µg/ml goat anti-hamster antibody at 37 °C. 2 × 10⁵ cells were lysed in 500 µl of ice-cold Rap1 lysis buffer containing 10% glycerol, 1% Nonidet P-40, 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 5 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride, 1 mM orthovanadate, and 10 µg/ml each aprotinin and leupeptin for 30 min. Lysates were clarified by centrifugation at 15,000 × g for 20 min at 4 °C. Supernatant were incubated with 5 µg of GST-Raid6s-RBD fusion protein coupled to glutathione-Sepharose beads for 2 h at 4 °C. Beads were pelleted and rinsed three times with the lysis buffer, and the proteins were eluted from the beads with Laemmli sample buffer and separated by electrophoresis on a SDS-PAGE (12%), followed by transfer to polyvinylidene difluoride membranes. Affinity-purified activated Rap1 were detected by immunoblotting using anti-Rap1 antibody.

LFA-1 Clustering Assay—Purified CD4+ T cells from Cbl-b−/− and Cbl−/− lymphocytes were incubated in cold medium with 10 µg/ml anti-CD3e (clone 145-2C11) on ice for 30 min. Cells were then washed twice with cold medium and cross-linked by incubating with 50 µg/ml goat anti-hamster IgG antibody (Jackson Immunoresearch) at 37 °C for 40 min. Cells were allowed to settle on poly-l-lysine-coated glass coverslips and fixed with 3.7% paraformaldehyde for 20 min, washed with PBS, and blocked with 2% BSA/PBS for 30 min. Samples were stained with FITC-anti-LFA-1 antibody (Southern Biotechnology Associates, Inc). LFA-1 clustering was visualized by using a confocal microscope (Bio-Rad). At least 100 T cells were counted for cap formation in each experiment.

Metabolic Labeling and Pulse-Chase Experiment—The experiment was performed by following previously published procedures (14, 15). Briefly, primary lymph node T cells from wild-type and Cbl-b−/− mice were incubated at 5 × 10⁵ cells/well in 24-well plates in Dulbecco’s modified Eagle’s medium lacking methionine and cysteine, plus 5% dialyzed fetal bovine serum. The cells were stimulated with anti-CD3 (5 µg/ml each) and were then labeled for 1 h at 37 °C by adding 100 µCi/ml [³⁵S]methionine and [³⁵S]cysteine (ICN Biomedicals, Costa Mesa, CA).

**FIG. 1. Interaction of Cbl-b with Crk-L in murine primary T cells.** A, lymph node T cells from B6 mice were left untreated or stimulated with anti-CD3 (10 µg/ml) or anti-CD3 plus anti-CD28 (20 µg/ml) for 5 min. Cell lysates prepared from these cells were subjected to immunoprecipitation with anti-Cbl-b. The immunoprecipitates were resolved on SDS-PAGE and electrotransferred on blotting membrane. The upper part of the membrane was blotted with anti-phosphotyrosine mAb (PY-19, top panel) and the lower part with anti-Crk-L (middle panel). The upper part was reprobed with anti-Cbl-b (bottom panel). B, cell lysates from similarly treated cells as in A were subjected to immunoprecipitation with anti-Crk-L and immunoblotting with anti-Cbl-b (top panel). The same membrane was reprobed with anti-Crk-L (bottom panel). IP, immunoprecipitation.

The cells were then washed twice with Dulbecco’s modified Eagle’s medium and chased for different times in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Cells were harvested at indicated time and the cell lysates were probed with protein G-Sepharose for 30 min and were immunoprecipitated with anti-Crk-L or anti-TeBa. Immunocomplexes were resolved by SDS-PAGE, and the gels were dried and subjected to autoradiography. The radiolabeled protein bands were quantified by using an NIH Image 1.61 software.

**RESULTS**

Cbl-b Associates with Crk-L in Primary T Cells—We previously reported that stimulation of human Jurkat T cells induces tyrosine phosphorylation of Cbl-b and its association with Crk-L (11). To examine whether similar events occur in murine primary T cells, we incubated mouse lymph node T cells with 2C11 anti-CD3 antibody alone or plus anti-CD28. Anti-CD3 stimulation indeed caused tyrosine phosphorylation of Cbl-b, as revealed by blotting anti-Cbl-b immunoprecipitates with anti-phosphotyrosine antibody (Fig. 1A, top panel). Co-stimulation with anti-CD28 further enhanced Cbl-b tyrosine phosphorylation. Crk-L was detected in anti-Cbl-b immunoprecipitates from untreated or treated cells (Fig. 1A, middle panel). Equivalent amounts of Cbl-b were present in all the immunoprecipitates from untreated or treated cells (Fig. 1A, bottom panel). Similarly, when the cells were subjected to immunoprecipitation with anti-Crk-L antibody, Cbl-b was detected from samples treated with anti-CD3 antibody (Fig. 1B, top panel). The communoprecipitation was further increased in samples treated with anti-CD28 cosimulation. The data indicate that like in human Jurkat T cells, Cbl-b and Crk-L form a complex in murine primary T cells.

Cbl-b Promotes Crk-L Ubiquitination without Affecting Its Stability—The recent identification of Cbl family proteins as E3 ligases for its binding proteins prompted us to investigate whether Cbl-b also promotes Ub conjugation to Crk-L. To this end, we cotransfected Cbl-b, Crk-L, and Myc-Ub in Jurkat T cells. As shown in Fig. 2A, coexpression of Cbl-b with Crk-L...
resulted in the formation of high molecular smears in anti-Crk-L immunoprecipitates, when blotted with anti-Myc antibody, suggesting polyubiquitination of Crk-L. Of note, Ub conjugation to Crk-L was increased upon anti-CD3 stimulation. We previously described that mutation at the conserved Trp or Cys residues of Cbl-b RING finger domain abolishes its E3 ligase activity (9). To consist with this observation, we found that ubiquitination of Crk-L was also abrogated by coexpression with the Cbl-b WA or CA mutant (Fig. 2B). To determine a physiological role of Cbl-b in Crk-L ubiquitination, we examined whether Cbl-b deficiency affects Ub conjugation to Crk-L. Primary lymph node T cells from wild-type or Cbl-b−/− mice were either untreated or stimulated with anti-CD3 antibody. Stimulation of primary T cells induced ubiquitination of Crk-L in wild-type T cells (Fig. 2C). However, Ub conjugation to Crk-L was reduced in Cbl-b−/− T cells under the same stimulation conditions.

As previously described, ubiquitination of p85 of PI3K by Cbl-b does not cause p85 degradation (10). To examine whether Cbl-b affects the stability of Crk-L, we stimulated primary T cells from wild-type and Cbl-b−/− mice with anti-CD3 antibody for different time periods. It was found that Crk-L is a very stable protein (Fig. 3A). No apparent change in Crk-L protein level was observed even after 6-h stimulation. Cbl-b deficiency did not affect the protein level of Crk-L, as compared with wild-type T cells. The same membrane was blotted with antibodies against C3G, Zap-70, Fyn, Lck, and Grb2. There was no discernable difference in the protein levels of these proteins before or after anti-CD3 stimulation of T cells from wild-type and Cbl-b−/− mice. To further confirm that Cbl-b deficiency does not affect the protein stability of Crk-L, we performed pulse-chase experiments (14) in which primary T cells were labeled first with [35S]methionine, and the stability of the radiolabeled Crk-L was traced by immunoprecipitation at different time intervals after initial labeling. We found that the half-life of Crk-L was quite similar in both cell populations (Fig. 3B). As a positive control, we traced the half-life of IκBα, a well known protein that goes through proteasome-dependent degradation. IκBα has a very short half-life, which was not affected by Cbl-b deficiency (Fig. 3B, bottom panel). The data reinforce our previous observation that Cbl-b exerts its E3 ligase activity toward its substrates without apparent proteolysis (10).

A recent study documented that stimulation of T cells with anti-CD3 or anti-CD3 plus CD28 induces self-ubiquitination and subsequent degradation of Cbl-b (16). We found, however, that stimulation of primary T cells with anti-CD3 for longer time (9–18 h) resulted in an increase of Cbl-b protein in these cells (Fig. 3C). Costimulation with anti-CD28 did not reduce Cbl-b protein expression. The anti-CD3-induced Cbl-b expression was prominent, since blotting of the same lysates with other signaling molecules such as Cbl, Zap-70, Crk-L, or Lck did not show, or showed only slightly, changes in their protein amounts (Fig. 3C).

Enhanced Crk-L and C3G Association and Rap-1 Activation in Cbl-b−/− T Cells—Next, we investigated whether Cbl-b deficiency affects the adaptor’s function of Crk-L. Crk-L associates with C3G through the SH3 domain in Crk-L and the proline-rich sequences in C3G (12). We found that this associ-
cation was constitutive in primary T cells, without obvious change upon TCR stimulation (Fig. 4A, top panel). However, the interaction between Crk-L and C3G was markedly increased in Cbl-b−/− T cells, under both resting and anti-CD3 stimulated conditions. It was also reported that Crk-L associates with Zap-70 in an activation-dependent manner in Jurkat T cells (17). Similar results could be observed in primary T cells upon anti-CD3 stimulation (Fig. 4A, middle panel). This activation-dependent interaction between Crk-L and Zap-70 did not change in Cbl-b−/− T cells. This change in Crk-L-C3G association in Cbl-b−/− T cells was not due to the change in protein levels of Crk-L or C3G, since blotting of the cell lysates with respective antibodies showed equivalent amounts of Crk-L and C3G proteins (Fig. 3B).

The increased interaction between Crk-L and C3G may affect the guanine exchange factor activity of C3G. It is known that C3G is a specific guanine exchange factor for Rap1 (12). Rap1 is present in a GDP-bound form, and when activated, becomes a GTP-bound form. To examine the Rap1 activation in T cells, we employed a pull-down assay in which only the activated Rap1 can be recognized by a GST fusion protein containing RBD (18). As shown in Fig. 4C, GST-RBD specifically precipitates Rap1 from primary T cells. Stimulation of the cells with anti-CD3 increased the amounts of activated Rap1. GST-RBD precipitated more Rap1 in resting Cbl-b−/− T cells, in comparison with wild-type T cells and the activated Rap1 was further increased upon anti-CD3 stimulation in Cbl-b−/− T cells. Blotting the cell lysates with anti-Rap1 antibody showed similar amounts of Rap1. The result suggests that Cbl-b is a negative regulator for Rap1 activation.

**Increased LFA-1 Activity in Cbl-b−/− T Cells**—A recent study using Rap1 transgenic mice showed that Rap1 positively regulates cell adhesion through activating LFA-1 activity in T cells (19). The increased Rap1 activation in Cbl-b−/− T cells prompted us to examine whether Cbl-b deficiency affects Rap1-mediated LFA-1 activation. To test this possibility, we examined the cell adhesion of primary T cells from wild-type and Cbl-b−/− mice to plate-bound ICAM-1-Fc protein. We found that T cells from Cbl-b−/− mice displayed increased attachment to ICAM-1-Fc, but not to BSA, as compared with wild-type T cells under both resting and anti-CD3 stimulated conditions (Fig. 5A). The difference in ICAM-1 binding is not due to the differential cell surface expression of LFA-1, as revealed by the equivalent amounts of anti-LFA-1 staining in T cell population.

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**Fig. 3.** Cbl-b does not affect the protein expression of its binding proteins. **A**, primary T cells were stimulated with anti-CD3 for different time intervals, and the cell lysates were blotted with indicated antibodies. **B**, primary T cells were labeled with [35S]methionine for 1 h, and the labeled cells were washed and incubated for indicated time periods. Cell lysates were immunoprecipitated with anti-Crk-L (upper panel) or anti-IκBα (bottom panel), and the radiolabeled proteins were detected by autoradiography. The numbers below each lane represent the relative intensity of the radiolabeled Crk-L or IκBα. **C**, primary T cells from wild-type mice were stimulated with anti-CD3 or anti-CD3 plus anti-CD28 for the time periods as indicated, and the cell lysates were blotted with antibodies as indicated.

**Fig. 4.** Crk-L and C3G interaction and Rap1 activation in primary T cells. **A**, lymph node T cells from wild-type or Cbl-b−/− mice were stimulated with anti-CD3 for the indicated time periods. Cell lysates were incubated with anti-Crk-L antibody, and the immunoprecipitates were blotted with anti-C3G (top panel) or Zap-70 (middle panel). The membrane was reprobed with anti-Crk-L (bottom panel). **B**, aliquots of cell lysates from A were blotted with antibodies as indicated. **C**, lysates from similarly treated cells as in A were incubated with GST-RBD fusion protein (5 μg/ml), and the precipitates were blotted with anti-Rap1. The same membrane was reprobed with anti-GST antibody. Aliquots of the same cell lysates were blotted with anti-Rap1 or Cbl-b. **IP**, immunoprecipitation.
tions from control and Cbl-b−/− mice (Fig. 5B). To further confirm that Cbl-b deficiency affects the binding ability of LFA-1 to ICAM-1, we performed FACS analysis on wild-type and Cbl-b−/− T cells using ICAM-1-Fc as a probe. The numbers of ICAM-1-Fc positive T cells were increased in both resting and anti-CD3 stimulated Cbl-b−/− T cells (Fig. 5C). Thus, Cbl-b deficiency increases LFA-1 activity, most likely through regulating Rap1 signaling.

Increased LFA-1 Clustering in Cbl-b−/− T Cells—It is established that engagement of T cells with antigen-presenting cells in the presence of antigenic peptide induces membrane clustering of TCR complex and accessory molecules (20, 21). LFA-1 is one of such molecules that are recruited to the membrane microdomain, which is critical for T cell activation and adhesion (22). The increased binding of Cbl-b−/− T cells to ICAM-1 suggests that Cbl-b deficiency may also affect LFA-1 clustering. To investigate this possibility, we examined the formation of LFA-1 aggregates in purified CD4+ T cells by cross-linking of the TCR with hamster anti-CD3 antibody, followed by secondary anti-hamster antibody. Although resting T cells showed uniformed staining to anti-LFA antibody around the cell surfaces, cross-linking of TCR resulted in the formation of LFA-1 clustering on T cell surface (Fig. 6A). When compared with wild-type T cells, Cbl-b−/− T cells showed more intense LFA-1 patches under anti-CD3 stimulated conditions. Under anti-CD3-stimulated condition, about 50% of the total T cells showed LFA-1 clustering in wild-type T cells (Fig. 6B). The number of LFA-1 patches was increased to 80% under the same stimulated conditions. The result suggests that Cbl-b is involved in T cell adhesion by regulating LFA-1 aggregation and activation.

**DISCUSSION**

In the present study, we presented data showing that Cbl-b acts as an E3 Ub ligase for Crk-L. Cbl-b promotes ubiquitination of Crk-L without affecting its stability. Rather, Cbl-b deficiency results in an increased association between Crk-L and C3G, which leads to augmented activation of Rap1, and subsequent enhancement of LFA-1 activation. The results suggest that Cbl-b plays a negative role in Crk-L-C3G signaling pathway in response to TCR stimulation.

We have previously demonstrated that Cbl-b regulates p85, the regulatory subunit of PI3K in an E3 ligase-dependent, proteolysis-independent manner (9, 10). Cbl-b-induced ubiquitination of p85 modulates its association with upstream molecules such as TCR subunits and CD28. The increased association between Crk-L and C3G in Cbl-b−/− T cells, as demonstrated in the present study, most likely results from the reduced Crk-L ubiquitination by Cbl-b deficiency, and the data further supports a notion that the Ub ligase activity of Cbl-b is uncoupled to the proteasome-dependent degradation of its substrates. At present, the molecular mechanisms by which Ub conjugation to a substrate modulates its biological function remain unclear. We propose that by helping tagging Ub to a
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substrate, Cbl-b may cause a structural change of its substrates, which may lead to a hindrance of their capability to recruit other binding molecules. It should be noted that the modification of Crk-L and C3G binding in Cbl-b−/− T cells is specific, since Cbl-b deficiency does not affect the association of Crk-L with Zap-70. It can be postulated that Ub conjugation to Crk-L may reside in the SH3 domains, which are required for C3G binding, not in the Zap-70-interacting SH2 domain.

It was recently reported that Cbl-b goes through self-ubiquitination and subsequent degradation, which is proposed to be a mechanism for Cbl-b-mediated T cell regulation (16). Although the present study did not address this issue directly, we found that stimulation of primary T cells with anti-CD3 did not affect the stability of Cbl-b. In contrast, anti-CD3 stimulation of primary T cells for longer period (9–18 h) increased the protein expression of Cbl-b, but not its homologue, Cbl. In addition, further costimulation with anti-CD28 did not cause a decrease of Cbl-b protein amount in these cells. Thus, our result does not support a critical role of Cbl-b self-ubiquitination in T cell regulation. The increased protein expression of Cbl-b in T cells after longer time stimulation may instead point out a novel negative feedback mechanism, i.e., TCR engagement not only triggers Cbl-b-mediated ubiquitination pathway to help terminate the activation signal, but also increases Cbl-b protein expression to counter-balance the sustained T cell activation. This mechanism may be particularly important in the development of autoimmune diseases in which a chronic and sustained T cell activation is required for the breakdown of T cell tolerance. To support this hypothesis, it was reported that Cbl-b deficiency results in spontaneous autoimmune or increased immune response to autoantigens (7, 8).

Previous studies have documented a positive role of Cbl in Crk-L-C3G-mediated signaling (13, 24). In these studies, Cbl was proposed to be an adaptor protein, which helps recruit Crk-L and C3G to form an activation complex for Rap1. In Jurkat T cells, it was shown that engagement of TCR induces Fyn activation and subsequent Cbl phosphorylation, which leads to Cbl-Crk-L association. Since Crk-L constitutively associates with Zap-70 and Fyn, this interaction induces increased immune response to autoantigens (7, 8).

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