Cbl-b: Roles in T Cell Tolerance, Proallergic T Cell Development, and Cancer Immunity

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Introduction

Protein ubiquitination is one of fundamental regulatory post-translational modifications regulating intracellular signaling and plays potent roles in regulating a variety of signals in both innate and adaptive immune cells. The selectivity of the ubiquitin-26S proteasome system for a particular substrate protein relies on the interaction between an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase. The unique feature of E3 ubiquitin ligases is to recognize substrate proteins and target them for ubiquitination [1]. Importantly Cbl-b is the only member of the Cbl family which has been documented to be crucial for T cell tolerance. In this brief review, we summarize the major work that we and others have carried out investigating Cbl-b in T cell tolerance and autoimmunity, proallergic T cell development, and cancer immunity.

Cbl-b in T cell tolerance and Autoimmunity

The genetic proof of the in vivo biological function of Cbl-b comes from gene targeting experiments in mice. Drs. Hua Gu and Penninger groups generated Cblb⁻/⁻ mice independently in 2000. Both groups found that Cblb⁻/⁻ mice are highly susceptible to autoimmunity [3, 4], suggests that Cbl-b plays a crucial in T cell tolerance induction.
b in maintaining T cell tolerance, Cblb−/− mice are highly susceptible to experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA), and autoimmune diabetes [4, 8, 15]. These data are further supported by the evidence that Cbl-b polymorphisms or mutations have been identified in patients with multiple sclerosis and lupus [16, 17], although the cellular and molecular mechanisms for Cbl-b in the development of these diseases remain to be determined.

As mice lacking Cbl-b are highly susceptible to Th17-mediated autoimmune diseases such as EAE and CIA, it was thought that Cbl-b might directly regulate Th17 cell differentiation. However, our recent study suggests that Cbl-b deficiency does not lead to a biased Th17 cell differentiation program [18]. Therefore, the increased Th17 responses observed during EAE development may not result from T cell-intrinsic loss of Cbl-b, but rather from the loss of Cbl-b in innate immune cells. It is envisioned that Cbl-b deficiency in innate immune cells, such as dendritic cells and macrophages, leads to heightened production of pro-inflammatory cytokines which promote Th17 cell development. This notion is supported by evidence that Cbl-b deficiency results in increased pro-inflammatory cytokine production by splenocytes upon LPS stimulation [19]. Therefore, it is important to use cell-type-specific Cblb knockout strain to dissect the role of Cbl-b in T cells vs. non-T cells.

Cbl-b in proallergic T cell development and allergic airway inflammation

The finding that Cbl-b was not required for Th17 cell differentiation was surprising. Using adoptive transfer of naïve CD4+ T cells from wild type (WT) and Cblb−/− mice into Rag1−/− recipients, followed by immunization with cardiac myosin peptide in Complete Freund’s adjuvant (CFA), we found that T cell-intrinsic loss of Cbl-b may not account for heightened myocarditis (our unpublished data) in a mouse model which has been shown to be mediated by Th17 [20, 21].

To further understand the role of Cbl-b in Th cell differentiation, we performed extensive in vitro Th cell differentiation assays. Interestingly, although the in vitro generation of Th1 and Th17 cells are comparable between WT and Cblb−/− T cells, Th2 and Th9 cell differentiation is significantly higher in Cblb−/− mice compared to WT controls [18], suggesting that Cbl-b inhibits Th2 and Th9 cell differentiation. To demonstrate the in vivo biological relevance of this observation we utilized a well-established mouse model of allergic asthma which has been shown to be mediated by both Th2 and Th9 [22]. As expected, mice deficient for Cbl-b display severe airway inflammation accompanied with heightened Th2 and Th9 cytokines in bronchoalveolar lavage fluid (BAL), and increased IgE in the serum [18]. These data strongly support that Cbl-b

Consistent with this notion, we demonstrated that CD28 costimulation potentiates Cbl-b self-ubiquitination and degradation, whereas CTLA-4-B7 interaction induces Cbl-b expression [5, 6]. As both CD28 and CTLA-4 are critical for peripheral T cell tolerance, our findings strongly support that Cbl-b is one of the key molecules involved in T cell tolerance induction. In support of this, Cbl-b has been shown to be required for T cell anergy induction in vitro and in vivo [7, 8]. Cbl-b was suggested to target PLC-γ1 and PKC-θ for ubiquitination in anergic T cells [7, 8], although it is unclear how Cbl-b specifically induces PLC-γ1 and PKC-θ ubiquitination in anergic T cells since Cbl-b associates with PKC-θ and possibly PLC-γ1 in naïve T cells upon TCR/CD28 stimulation [9, 10]. Interestingly, Cbl-b appears to be important for the conversion of naïve CD4+CD25− T cells into CD4+Foxp3+ regulatory T cells induced by TGF-β in vitro, and for the peripheral generation of CD4+Foxp3+ regulatory T cells [11-13], a process that is regulated by the T cell activation threshold via an Akt-2-dependent mechanism [13]. In keeping with this observation, we found that Cblb−/− T cells display heightened Pten inactivation, and hyper-activation of Akt [14]. Therefore, Cbl-b regulates T cell tolerance via multiple mechanisms (Fig. 1). In support of the importance of Cbl-

**Figure 1. Model of Cbl-b in T-cell activation and tolerance.** Upon TCR stimulation, Pten is inactivated via Nedd4 which targets Pten for K63-linked polyubiquitination, and this process is inhibited by Cbl-b. Inactivation of Pten leads to the accumulation of PIP3 which recruits PDK-1, Vav-1 and Akt to the plasma membrane via its interaction with the PH domains within these molecules. Therefore, Vav1 links PKC-θ to PDK-1, the former coupling IKKs to the CBM complex. Activated Akt also facilitates the formation of the CBM complex possibly by phosphorylating CARMA1. Thus, Cbl-b inhibits NF-κB activation via PKC-θ and Akt. One of the important outcomes for Akt activation is that Akt-2 can phosphorylate Foxo1/3a which excludes them from the nucleus, thus inhibiting Foxp3 expression (not shown). In anergic T cells, Cbl-b targets PLC-γ1 and PKC-θ for ubiquitination, thus inhibiting T cell anergy induction. The expression of Cbl-b in T cells is controlled by CD28 and CTLA-4. CD28 costimulation induces Cbl-b ubiquitination and proteasomal degradation, while CTLA-4-B7 interaction induces Cbl-b expression.
suppresses proallergic Th2 and Th9 development and allergic airway inflammation. At the molecular levels Cbl-b selectively binds to Stat6, a transcription factor which is critical for the development of both Th2 and Th9, and targets it for polyubiquitination (at K108 and K398) which leads to the proteasome-mediated degradation. In the absence of Cbl-b, Stat6 ubiquitination and degradation is impaired, which results in heightened Th2 and Th9 responses and allergic airway inflammation. Introducing Stat6 deficiency into Cblb-/- mice abrogates hyper-Th2 responses but only partially attenuates Th9 responses, suggesting that Cbl-b regulates Th2 cell differentiation via a Stat6-dependent mechanism but regulates Th9 cell differentiation via both Stat6-dependent and -independent mechanisms.

Cbl-b in anti-tumor immunotherapy

The enhanced T cell responses in the absence of Cbl-b prompted scientists to determine whether Cbl-b regulates anti-tumor immune responses. Deletion of Cbl-b confers spontaneous in vivo rejection of tumor cells that express human papilloma virus antigens [24]. Introduction of the Cbl-b deficiency into tumor-prone ataxia telangiectasia mutated–deficient mice markedly reduces the incidence of spontaneous thymic lymphomas [25]. Moreover, Cblb-/- mice develop significantly fewer ultraviolet B (UVB)-induced skin malignancies and reject UVB-induced skin tumors [24]. Cblb-/- mice also reject transplanted E.G7 and EL4 lymphomas [25]. The enhanced anti-tumor activity in
the absence of Cbl-b is likely mediated by hyper-activation of CD8+ T cells which are resistant to the regulation by regulatory T cells or TGF-β [24, 25]. In support of these findings, abrogating Cbl-b in effector CD8+ T cells improves the efficacy of adoptive therapy of leukemia in mice [26].

Subcutaneous implantation of TC1 tumor cells, as well as induction of spontaneous tumors by UV irradiation, leads to a significantly delayed outgrowth of tumors in CblbΔ/Δ mice when compared to WT mice [24]. Interestingly, subcutaneous injection of TC-1 tumor cells into Cblb−/−/Rag2−/− mice, which lack T and B cells, causes a significant delay in tumor growth compared to Cblb+/+Rag2+/+ littermates [27], suggesting that innate cells are involved in this process. Further analysis revealed that inactivation or deletion of Cbl-b licenses the natural killer (NK) NK cells to spontaneously reject metastatic tumors. The heightened anti-tumor activity by NK cells lacking Cbl-b is mediated by the TAM tyrosine kinase receptors Tyro3, Axl, and Mer. Treatment of WT NK cells with a newly-developed small molecule TAM kinase inhibitor confers therapeutic potential, efficiently enhancing anti-metastatic NK cell activity in vivo [27]. Therefore, Cbl-b appears to be a therapeutic target for cancer immunotherapy.

Conclusions

During the last 14 years, significant progress has been made towards our understanding of E3 ubiquitin ligase Cbl-b in T cell tolerance, autoimmunity, proallergic T cell development and allergic asthma, as well as cancer immunotherapy. These studies collectively indicate that Cbl-b is an excellent drug target for autoimmune disease, allergic asthma, and cancers. Future studies will be required to identify small molecules that could modify the enzymatic function of Cbl-b.

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Conflict of Interests

The authors declare that there is no conflict of interests.

References


