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The carboxy-terminal region of CD5 is required for c-CBL mediated TCR signaling downmodulation in thymocytes

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ABSTRACT

CD5 functions as a negative regulator of TCR signaling during thymocyte development, however, the molecular mechanisms involved in this process remain elusive. A key molecule involved in the down modulation of TCR signaling is c-Cbl, an ubiquitin ligase that physically associates with CD5. Crosslinking of TCR in thymocytes leads to ubiquitylation and lysosomal/proteasomal degradation of TCR downstream signaling effectors and CD5 itself. The present report shows that co-engagement of CD3 with CD5 enhanced c-Cbl phosphorylation, which was not affected by the deletion of the pseudo-ITAM domain of CD5, the putative binding site for c-Cbl. However, amino acids present in the carboxy-terminal region of CD5, were necessary for this effect, indicating that ITAM-independent sites were involved in the interaction of c-Cbl with CD5. The carboxy-terminal region of CD5 was also required for Vav degradation, a well-known target for c-Cbl-dependent ubiquitylation. These results support the notion that the distal cytoplasmic domain of CD5, including Y463, plays a relevant role in the downmodulation of TCR signals in thymocytes via c-Cbl.

1. Introduction

CD5 is a 67 kDa glycoprotein, expressed on thymocytes, peripheral T cells and a subset of B cells (B1-a). It physically associates with the TCR and BCR, where it negatively modulates the activation and differentiation signals transduced by these receptors [1]. Moreover, CD5 also plays a role in protection from activation-induced cell death and in the recognition of pathogen associated molecular patterns (PAMPS) present on fungal surfaces [2].

Although the long cytoplasmic tail of CD5 lacks intrinsic catalytic activity, it is well adapted to signal transduction, as it contains four tyrosine phosphorylation sites, including an immunoreceptor tyrosine-based (ITAM)-like motif (pseudo-ITAM) and an immunoreceptor tyrosine-based inhibitory (ITIM)-like motif (pseudo-ITIM), as well as multiple potential serine and threonine phosphorylation sites [1]. Upon TCR engagement, the CD5 cytoplasmic tail becomes phosphorylated leading to the recruitment of several signaling molecules including $p56^{lck}$, TCRζ chain (p21) and ZAP70, PI3K, Vav, Rac1, the ubiquitin ligase c-Cbl, Ras GTPase-activating protein (RasGAP) and the tyrosine phosphatase SHP-1 (reviewed in [1]). Additionally, CD5 ligation allows the activation of Casein Kinase II (CK2), which is constitutively associated to its cytoplasmic tail, as well as the activation of protein kinase C by a phosphatidylcholine (PC)-specific phospholipase C-dependent pathway [1].

Initial *in vitro* experiments showed that CD5 acts as a costimulatory molecule by enhancing TCR/CD3 mediated responses, however, the function of CD5 as a negative regulator of TCR signaling emerged from the analysis of $CD5^{-/-}$ mice (revised in [2]). In this context, CD5 was found to regulate the threshold of positive and negative selection [3] and, more recently, the generation of thymic nTregs [4].

Although the molecular mechanisms involved in CD5-dependent negative signaling still remain elusive, several independent studies have implicated the membrane-proximal ITIM motif [5], the pseudo-ITAM motif encompassing Y429 and Y441 [6] as well as the recruitment and activation of negative regulators such as SHP-1, RasGAP, c-Cbl and CK2 (revised in [1,2]). In addition, a recent report, has proposed an inhibitory effect of CD5, through the phosphorylation of the negative regulatory Y531 of Fyn [7].

c-Cbl is an ubiquitin ligase of 120 kDa highly expressed in the thymus. Upon TCR stimulation, c-Cbl is rapidly phosphorylated on tyrosine residues, and targets activated proteins, including

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Syk, ZAP-70, Fyn, Lck, Vav, p85 PI3K, Crk and Grb2 (reviewed in [8]) and CD5 [9], for proteasomal and/or lysosomal degradation through its RING finger domain. C-Cbl plays a critical role TCR signaling downregulation during thymocyte development. Indeed, T cell signaling c-Cbl$^{-/-}$ mouse thymocytes is enhanced and results in increased positive selection of CD4+ cells, indicating that c-Cbl is a negative regulator of thymic positive selection [10].

c-Cbl can associate with CD5 in stimulated thymocytes [11] and has been recently shown to induce its ubiquitylation, leading to downmodulation of surface CD5 expression in developing thymocytes [9]. However, the interaction site for this association remains unknown. Thus, we hypothesized that c-Cbl phosphorylation and/or association with CD5 would contribute to the downregulation of TCR signaling during thymocyte development and that specific sequences within the cytoplasmic tail of CD5 may be important for this effect.

2. Materials and methods

2.1. Mice

BALB/c mice were purchased from Jackson Laboratories and maintained in the mouse facility at the Instituto de Investigaciones Biomédicas, UNAM, in accordance with Institutional guidelines. Four to six-week-old mice were used in the experiments.

2.2. Reagents and antibodies

Anti-mouse CD5-PE (anti-mCD5) (53-7.3), anti-phospho-c-Cbl-Alexa 647 (anti-pY700) and Streptavidin-PE, were purchased from BD Biosciences (San Jose, CA). Biotin-labeled anti-mCD3 (145-2C11) and anti-mCD5 (53-7.3) were purchased from Biolegend (San Diego, CA). Monoclonal anti-c-Cbl (A-9), polyclonal anti-c-Cbl (C-15) and polyclonal anti-Vav (C-14) antisera were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phosphotyrosine (4G10) was obtained from Millipore, Millipore Corporation (Bedford, MA). The following secondary reagents were used: goat anti-mouse IgG-FITC (ZyMax™ Grade, Invitrogen, Carlsbad, CA); (HRP)-labeled anti-mouse Ig (GE Healthcare, UK) and goat anti-rabbit IgG (Invitrogen). Mouse anti-human CD5 (anti-hCD5) mAb (Cris-1) and polyclonal antisera against the extracellular and the intracytoplasmic regions of human CD5 were previously described [12]. Immunopure® streptavidin (Pierce Biotechnology, Rockford, IL), was used for crosslinking of biotin-labeled antibodies.

2.3. Cell lines, transfection and cell culture

10×10^6 EL-4 cells were stably transfected with 10 μg of NdeI-linearized expression vectors. Constructs containing the cDNAs for wild-type human CD5 (hCD5.WT) or hCD5 cytoplasmic tail mutants [13] were used (Fig. 1B). G418 resistant stable clones were selected and enriched for mCD3^{high} hCD5^{high} by cell sorting, using a FACSAria cell sorter (BD Biosciences).

2.4. Cell stimulation and phosphorylation assays

Clones were stimulated with biotin-labeled anti-mCD3 antibodies alone or in combination with anti-mCD5 or anti-hCD5 (10 μg/ml each) and crosslinked at 37 °C for 3 min with streptavidin (20 μg/ml). Pervanadate (PV; 100 nM) treatment for 5 min at 37 °C was used as positive control. Phosphorylation of c-Cbl (p-c-Cbl) was detected by flow cytometry after intracellular staining with anti-pY700 antibody, following a slightly modified phospho-flow (BD Biosciences) protocol [4]. Murine thymocytes and the parental EL-4 cell line were used as positive controls. Data was analyzed using FlowJo software® (Tree Star, Inc., Ashland, OR).

2.5. Immunoprecipitation and Western Blotting (WB)

Total lysates of 35×10^6 stimulated or unstimulated cells were obtained and immunoprecipitated with anti-c-Cbl or anti-Vav and Protein G Sepharose and Western blot analysis was performed as previously described [12].

2.6. Densitometry

Densitometric analysis was performed directly on scanned images using ImageJ 1.45 software (NIH). Signals obtained from the analysis were expressed in arbitrary units.

2.7. Statistical analysis

Unpaired two-tailed Student's *T* test was used to calculate statistical significance. $p < 0.05$ were considered as significant.

3. Results and discussion

3.1. CD5 co-crosslinking enhances CD3-dependent c-Cbl phosphorylation in murine thymocytes and thymoma EL-4 cells

Previous reports have shown that c-Cbl, a well known downmodulator of TCR signaling, associates to CD5 and becomes phosphorylated after CD5 crosslinking thereby mediating CD5 degradation [9,11] To determine the contribution of CD5 in c-Cbl-dependent downmodulation of TCR signaling, we first analyzed the phosphorylation of c-Cbl (Y700) following crosslinking of CD3 alone or in combination with CD5 on murine thymocytes from 4 week-old mice and EL-4 thymoma cells. A significantly enhanced phosphorylation of c-Cbl (p-c-Cbl) was observed in thymocytes and EL-4 cells when co-crosslinked with anti-mCD5 compared to anti-mCD3 alone (Fig. 1A). These results indicate that CD5 co-ligation increases p-c-Cbl at a residue that is crucial for its role as ubiquitin ligase compared to engagement of CD3 alone, and support the notion that CD5 may contribute to c-Cbl mediated downmodulation of TCR signaling during thymocyte development [14].

3.2. The carboxy-terminal region of CD5 is required for enhanced c-Cbl phosphorylation in response to TCR co-crosslinking

Previously, it was reported that the inhibitory function of CD5 on TCR signaling during thymocyte development required functional integrity of the CD5 cytoplasmic tail, as demonstrated by the inability of a truncated CD5 transgene lacking the codons encoding the pseudo-ITAM and distal sequences of CD5, to rescue the CD5$^{-/-}$ phenotype [3]. This led us to test whether the pseudo-ITAM domain and/or the distal amino acids of CD5 were also involved in promoting enhanced TCR-mediated phosphorylation of c-Cbl. To address this question we took advantage of the high homology between the mouse and human CD5 cytoplasmic tail amino acid sequences (51%) and used human wild-type (hCD5.WT) and cytoplasmic tail deletion mutants (Fig. 1B) [13] to identify the domains involved in hCD5-mediated c-Cbl phosphorylation: hCD5.K384^{STOP} lacking essentially all of CD5 cytoplasmic tail; hCD5.H449^{STOP} maintaining the pseudo-ITAM but lacking the distal S and Y in the carboxy-terminal region; and hCD5.ΔE418-L444 lacking only the pseudo-ITAM domain, which is considered the putative site for c-Cbl association [11]. These constructions

were transfected into EL-4 cells and stable clones were selected for equivalent expression of hCD5, mCD3 and mCD5, as detected by flow cytometry (not shown).

Analysis of p-c-Cbl in each clone demonstrated that EL-4 cells expressing either hCD5.WT or hCD5.ΔE418-L444, showed greater phosphorylation following co-engagement of mCD3 with hCD5 or mCD5 compared to only mCD3 (Fig 1C). The levels of c-Cbl phosphorylation observed for hCD5.WT or hCD5.ΔE418-L444 transfectants following co-engagement of hCD5 with mCD3 were similar to those achieved by co-ligation of endogenous mCD5 with mCD3. However, when comparing the relative increment of p-c-Cbl, the hCD5.ΔE418-L444 expressing EL-4 cells showed a significant higher level of p-c-Cbl ($p = 0.048$) compared to hCD5.WT ($p = 0.019$) following co-ligation of mCD3 and hCD5 (Fig. 1D). This difference could not be attributable to increased expression of mCD3 and hCD5 (data not shown).

The enhanced phosphorylation of c-Cbl in the hCD5.ΔE418-L444 transfectant compared to hCD5.WT may reflect the lack of recruitment of negative regulators of TCR signaling such as RasGAP, which is known to bind to the ITAM-like domain deleted in such a construct. Indeed, RasGAP was shown to be recruited to CD5 at the pYSQP–pYPAL (Y429–Y441) sequence following pervanadate stimulation of thymocytes [11]. Since the hCD5.ΔE418-L444 mutant lacks the putative binding site for c-Cbl, the elevated levels of p-c-Cbl following hCD5 co-stimulation were unexpected. We therefore hypothesized that the distal cytoplasmic domain of CD5 could mediate this property. Indeed, we found that the CD5-dependent enhancement of c-Cbl phosphorylation was absent in hCD5.H449^{STOP} expressing EL4 cells when co-stimulated with antibodies to mCD3 and hCD5 (Fig. 1C and D). However, co-ligation of mCD3 with endogenous mCD5 did enhance c-Cbl phosphorylation demonstrating no intrinsic defect within this transfectant. Predict-

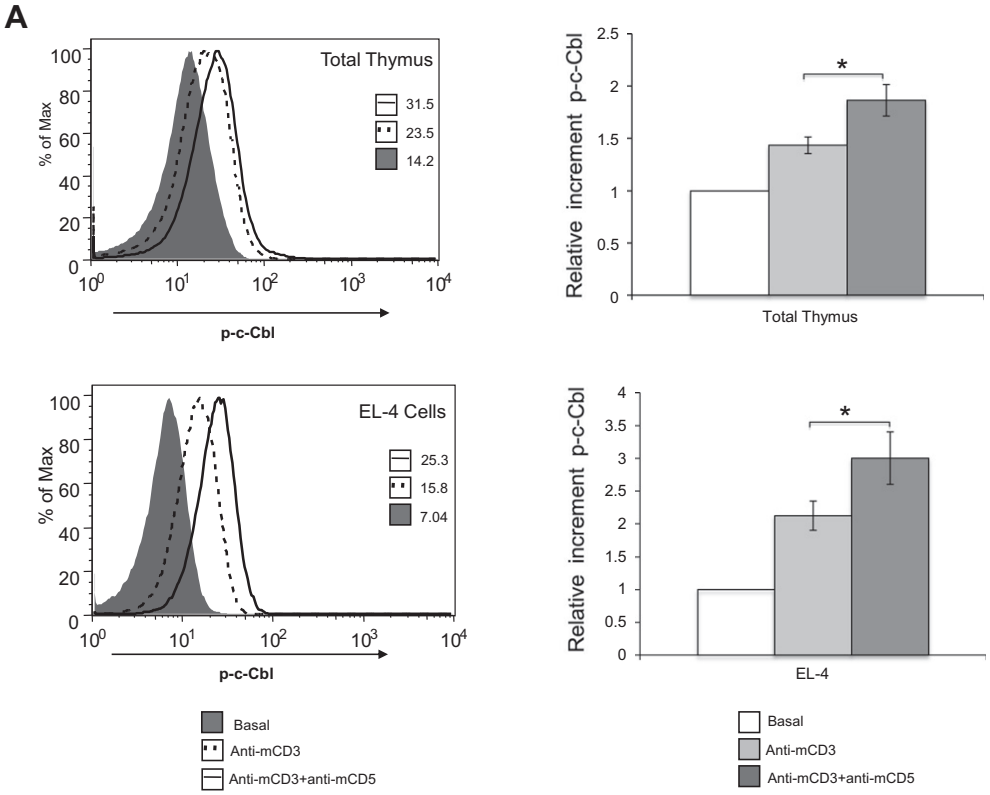

Fig. 1. The carboxy-terminal region of CD5 is required for increased phosphorylation of c-Cbl. (A) Total thymocytes and parental EL-4 cells were analyzed for intracellular p-c-Cbl by flow cytometry. A representative experiment is shown. The numbers indicate mean fluorescence intensity (MFI) values. The relative increment in p-c-Cbl, was calculated comparing the phosphorylation (MFI) obtained in the stimulated samples: anti-mCD3 or anti-mCD3 plus anti-mCD5 to unstimulated controls (basal). Data represent mean values ± standard error (SEM) ($n = 4$). *T*-test $^{*}p \leqslant 0.05$. (B) Schematic representation of the cytoplasmic region of wild-type human CD5 (hCD5) and cytoplasmic tail-mutated hCD5 molecules used in this work. (C) P-c-Cbl from stable EL-4 transfectant clones expressing wild-type or cytoplasmic tail-mutated hCD5 receptors either under basal conditions (medium alone) or following crosslinking with anti-mCD3 alone or in combination with anti-hCD5 or anti-mCD5. A representative experiment is shown. (D) Summary of experiments. Relative increment in p-c-Cbl shown was calculated as described above. Data represent mean values ± SEM ($n = 4$). *T*-test $^{*}p \leqslant 0.05$.

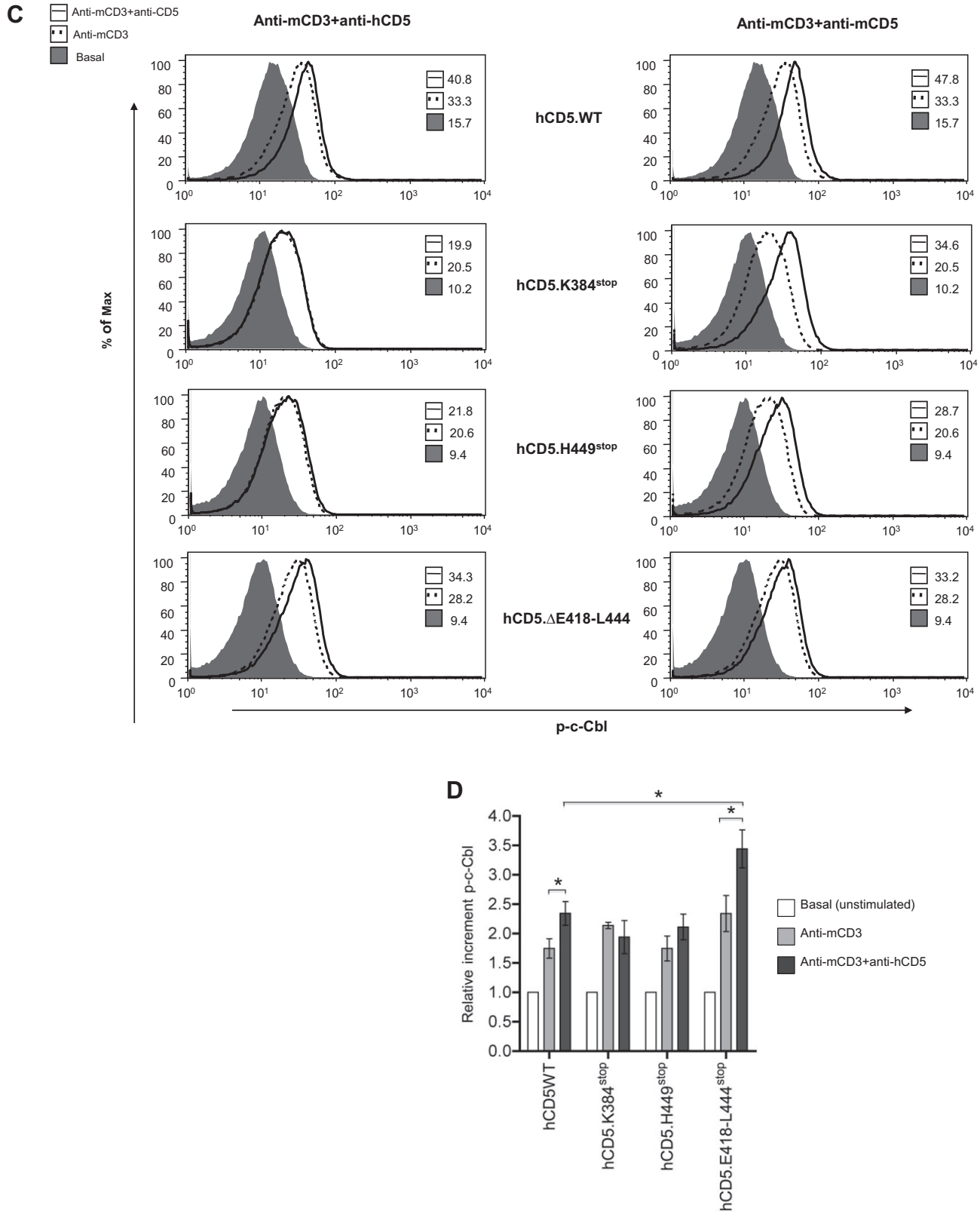

Fig. 1. (*continued*)

ably, anti-hCD5 did not augment the anti-CD3 induced c-Cbl phosphorylation in the hCD5.K384^{STOP} mutant (Fig. 1C and D).

The increased phosphorylation of c-Cbl in the hCD5.ΔE418-L444 mutant may alternatively be explained by the fact that the C-terminal region of CD5 is required for activation of Fyn kinase [15,16], which has been shown to be responsible for the phosphorylation of c-Cbl (reviewed in [8]). CD5 ligation reduces Fyn activity by increasing the phosphorylation on its inhibitory tyrosine (Y351)

and then promoting a complex with PAG-Csk [7], and inactive Fyn can recruit c-Cbl through its proline-rich region (reviewed in [17]). Upon CD3 crosslinking, Fyn will dissociate from the PAG-Csk and allow for the phosphorylation of c-Cbl. In this model CD5 may function as an "adapter" bringing c-Cbl and Fyn proximal to the TCR/CD3 receptor complex. Such a model would explain the inability of the hCD5.K384^{STOP} and hCD5.H449^{STOP} mutants to costimulate CD3-mediated p-c-Cbl, despite the later containing Y429 [11].

In the hCD5.H449^{STOP} mutant, Y429 and Y441 could allow the recruitment of other signaling molecules through their SH2 domain, including Lck [18], and PI3K [19] and possibly ZAP-70 [20] and mediate the indirect association of c-Cbl. However, co-crosslinking of hCD5 with mCD3 did not result in enhanced c-Cbl phosphorylation possibly due to RasGAP recruitment to the pseudo-ITAM [11]. One possible mechanism that could explain this result might be direct competition between RasGAP and c-Cbl, or alternatively the absence of the carboxy-terminal region, which is crucial for the recruitment of the activating proteins such as PI3K [19].

3.3. The carboxy-terminal region of human CD5 is required for the recruitment of phosphorylated c-Cbl

The conserved DNEY motif (Y429) of CD5 was shown to be involved in the interaction with c-Cbl [11]. However, it could not be ascertained whether the binding of c-Cbl to CD5 occurred through a direct or indirect association, as tyrosine-phosphorylated peptides corresponding to hCD5 pseudo ITAM sequence (pYSQP (Y429)–pYPAL (Y441)) and pYDLH (Y463) were unable to precipitate c-Cbl [11]. However, c-Cbl may be brought to CD5 by association with other SH2-SH3 containing molecules, as mentioned above. Recently, it has been demonstrated the association of c-Cbl with CD5 upon CD3 crosslinking, however, the specific site of interaction was not investigated [9].

In order to investigate the region of hCD5 involved in the association with c-Cbl, EL-4 transfectants were stimulated and the cell lysates immunoprecipitated with anti-c-Cbl and analyzed for associated tyrosine-phosphorylated proteins and hCD5 by WB. As illustrated by Fig. 2, p-c-Cbl co-immunoprecipitated with phosphorylated hCD5 in hCD5.WT and hCD5.ΔE418-L444 EL-4 transfectants after co-crosslinking of mCD3 and hCD5. c-Cbl associated with hCD5 only when cells were costimulated and not under basal conditions, confirming previous studies [9,11]. Recruitment of c-Cbl to hCD5 did not occur when cells were co-stimulated with anti-CD3 and anti-mCD5, thus confirming the specificity of the interaction. In contrast, in hCD5.H449^{STOP} expressing EL-4 cells c-Cbl associated only with unphosphorylated hCD5 under the same conditions. This result may imply that the presence of the pseudo-ITAM in hCD5.H449^{STOP} may downregulate TCR signaling through the interaction with negative regulators, such as RasGAP [11] or other yet unidentified signaling effectors, which may contribute to the unphosphorylated status of this hCD5 mutant. On this regard, it could be speculated that in the hCD5.H449^{STOP} mutant, RasGAP may associate to the SH2 domain of Lck involved in the binding to Y429. This would downregulate the Ras–Erk pathway, which has been shown to

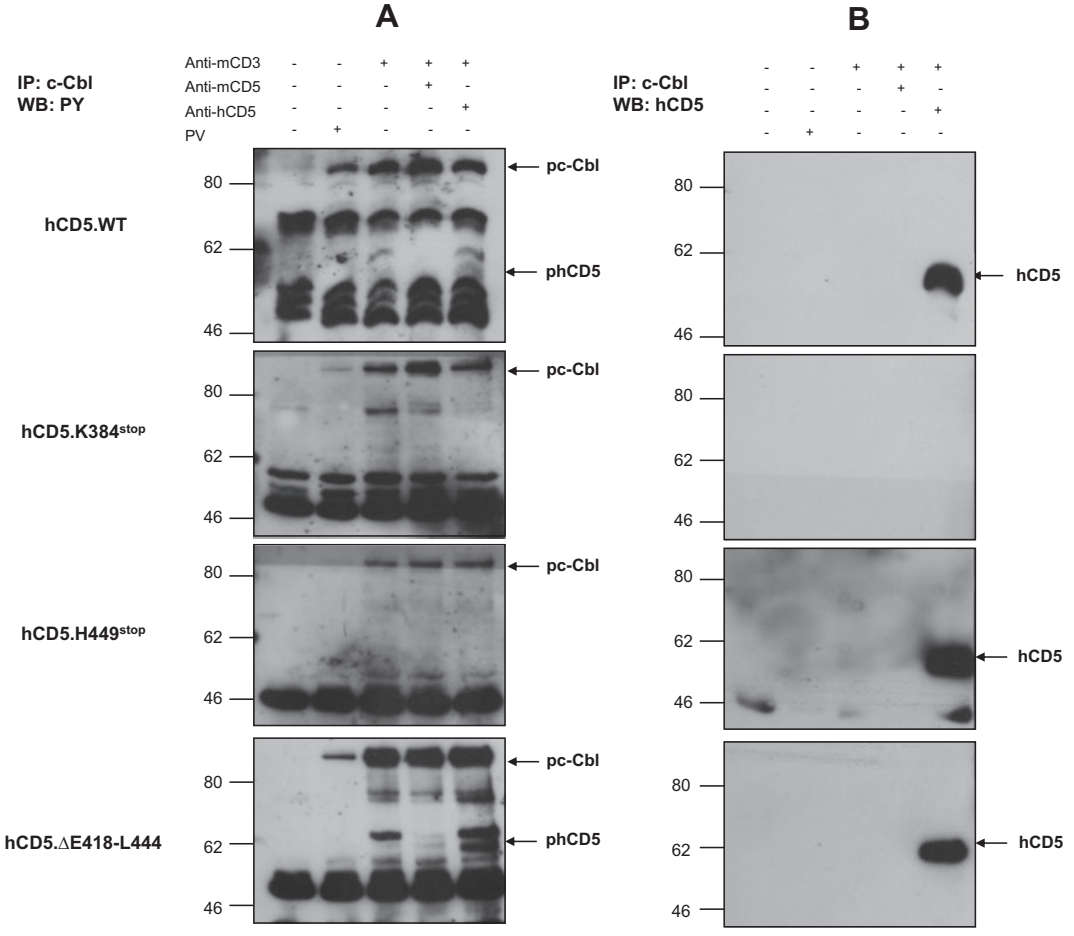

Fig. 2. Association of phosphorylated c-Cbl requires the carboxy-terminal region of hCD5. EL-4 cells were stimulated as described in Fig. 1, or with pervanadate (PV) as positive control. Cell lysates were immunoprecipitated overnight with anti-c-Cbl, immunoblotted with anti-pY (A) and re-probed with a polyclonal antiserum against extracytoplasmic region of hCD5 (B). A representative experiment is shown ($n = 3$).

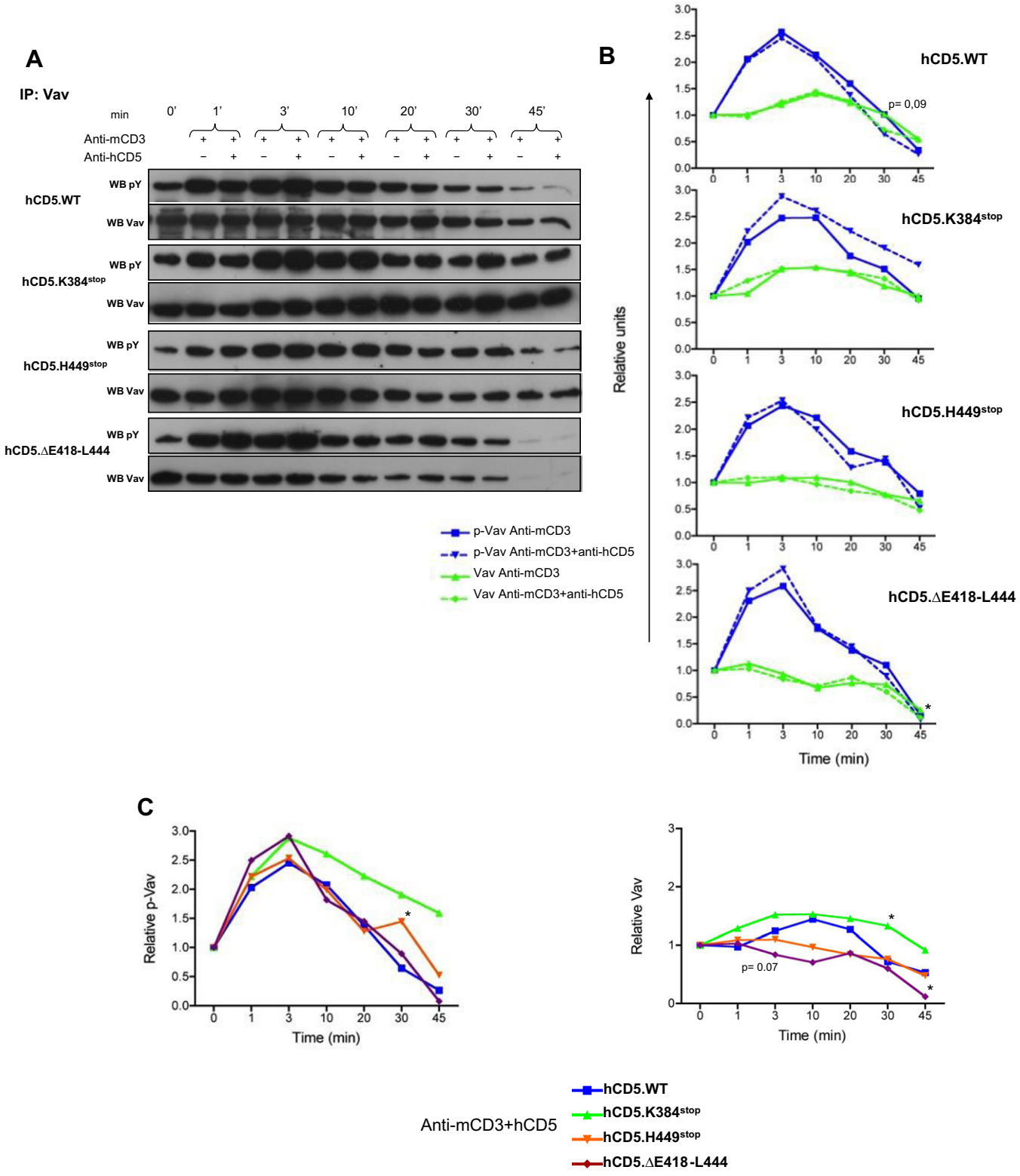

Fig. 3. Time course analysis of Vav phosphorylation and degradation in stable EL-4 transfectants. (A) Representative blots of EL-4 transfectants expressing WT and mutant hCD5 mutant molecules exposed to anti-mCD3 alone or in combination with anti-hCD5, at the indicated times. Total lysates immunoprecipitated with anti-Vav were probed for phosphotyrosine (upper blots) and total Vav (bottom blots) for each mutant. (B) The levels of phosphorylated Vav (p-Vav) and total Vav protein following ligation of mCD3 alone or in combination with anti-hCD5 were quantified by densitometry (arbitrary units), and values represent the relative increment compared to basal phosphorylation (value of 1). Data represent mean values ± SEM ($n = 3$). Asterisks indicate $^{*}p \leqslant 0.05$. (C) Comparative analysis of the levels of phosphorylated Vav (left) and total Vav (right) protein after co-cross linking of mCD3 and hCD5 for each clone.

phosphorylate Lck at Ser59 in response to TCR activation, thus interfering with its capacity to bind SHP-1. In this way, RasGAP association may favor SHP-1 mediated downregulation of TCR signaling [21] and possibly CD5 dephosphorylation.

As expected, there was no association of c-Cbl with hCD5.K384^{STOP} EL-4 transfectants, which lack most of the cytoplasmic tail of hCD5. Finally, the fact that there was no apparent co-immunoprecipitation of mCD5 in any of the transfectants

following either co-ligation of mCD3 and mCD5 or pervanadate stimulation is in accordance with a recent report showing that association of c-Cbl leads to ubiquitylation and degradation of CD5 [9]. Alternatively, the lack of association may be the result of a direct, but short-lived, interaction of c-Cbl with the DNEY sequence of mCD5 that may not be detectable by WB [11].

3.4. Recruitment of c-Cbl to the carboxy-terminal region of hCD5 results in increased Vav phosphorylation and degradation

Previous studies have shown that c-Cbl modulates the function of Vav during thymocyte development and T cell activation [22]. TCR/CD3 crosslinking leads to phosphorylation of c-Cbl at residue Y700 leading to the recruitment and ubiquitylation of Vav and its degradation by the proteasome [23]. To investigate whether the recruitment of c-Cbl observed in our mutants correlated with ubiquitin ligase activity, we analyzed the phosphorylation and degradation of Vav, which in turn may also be indirectly recruited to CD5 through its reported interaction with the p85 subunit of PI3K [24]. As illustrated in Fig. 3A and B, all transfectants showed phosphorylation of Vav (p-Vav) which peaked at 3 min and decreased around 10 min, returning to basal levels or lower by 45 min. However, the effect of hCD5 co-ligation on mCD3-mediated Vav phosphorylation and degradation differed among transfectants. In hCD5.WT transfected cells phosphorylation of Vav decreased more rapidly after hCD5 co-crosslinking compared to mCD3 alone. This fact was more evident at 30 min, correlating with a decrease in total Vav protein (Fig. 3B).

In the hCD5.K384^{STOP} transfectant, Vav phosphorylation in response to co-ligation was higher than of mCD3 alone (Fig. 3B). Interestingly, this effect was also observed in mCD3 crosslinking alone, which indicates that hCD5 can regulate TCR mediated signals, in the absence of antibody co-crosslinking (Fig. 3A and B).

On the other hand, analysis of the hCD5.H449^{STOP} transfectant, which lacks the carboxy-terminal region of hCD5, phosphorylation of Vav exhibited a bimodal curve following co-engagement of mCD3 and hCD5 (Fig. 3B). pVav levels peaked at 3 min and rapidly decreased followed by a second lower peak at 30 min. By 45 min the pVav levels reached basal levels. Notably, hCD5 co-ligation did not enhance mCD3-mediated Vav degradation. This result indicates that the pseudo ITAM is not necessary for the ubiquitin ligase function of c-Cbl, and correlates with the data shown above on c-Cbl phosphorylation and association (Fig. 2).

Finally, in hCD5.ΔE418-L444 transfectants, co-crosslinking of mCD3 and hCD5 resulted in reduced levels of phosphorylated Vav at 45 min compared to mCD3 alone (Fig. 3B).

By comparing Vav phosphorylation and degradation induced by mCD3 plus hCD5 co-crosslinking among clones expressing mutated and WT forms of hCD5 (Fig. 3C), it was observed that, as expected, the hCD5.K384^{STOP} mutant showed downregulation of pVav and or degradation of total Vav, but to a lower extent than hCD5WT, hCD5.H449^{STOP} and hCD5.ΔE418-L444. On the other hand, in the hCD5.H449^{STOP} mutant, levels of pVav significantly increased at 30 min (Fig. 3C), which may be explained by the reported negative regulatory role of the pseudo-ITAM [6] present in this mutant. These data also correlate with the lack of CD5-mediated costimulation of c-Cbl phosphorylation (Fig. 1C and D) and with the decreased degradation of Vav (Fig. 3C). By contrast, in the hCD5.ΔE418-L444 mutant, which lacks the pseudo-ITAM but contains the carboxy-terminal region of hCD5, co-ligation of hCD5 led to increased levels of phosphorylated Vav at 3 min compared to hCD5.WT (Fig. 3C), indicating a higher intensity signal, which correlated with a significant increase in the degradation of Vav at 45 min (Fig. 3C). These results indicate that recruitment of c-Cbl to the cytoplasmic region of hCD5 is important for the negative regulation of Vav, reducing Vav phosphorylation and inducing its degradation by the ubiquitin ligase activity of c-Cbl.

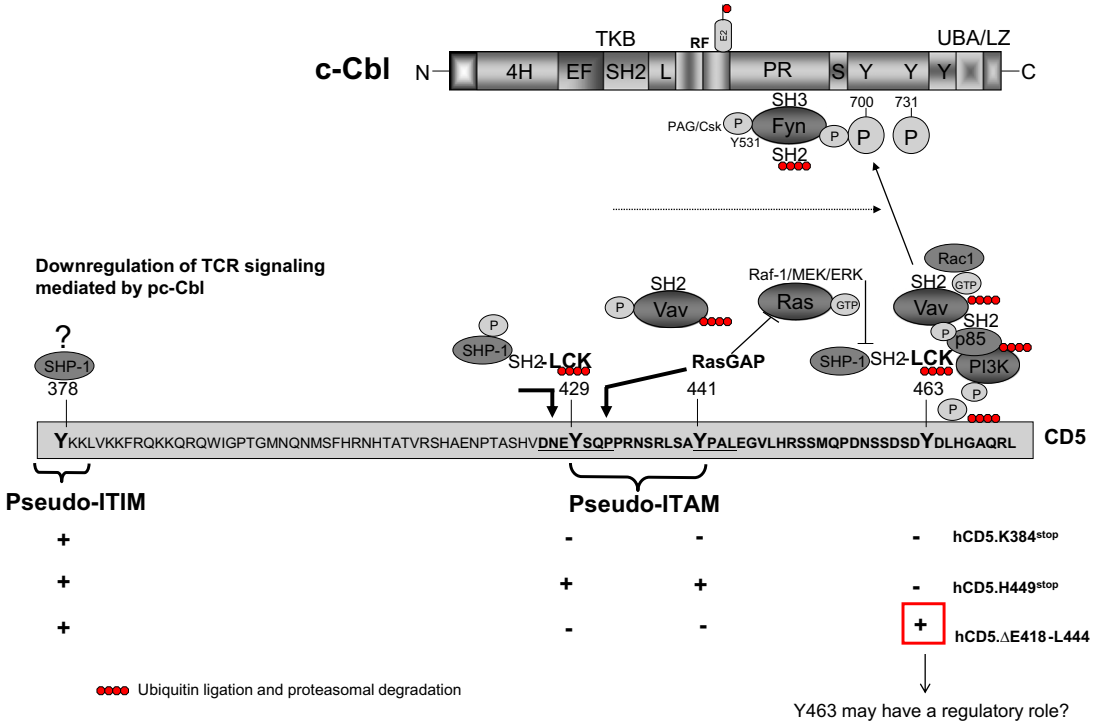

Fig. 4. Proposed model by which c-Cbl mediates CD5-dependent TCR downregulation in thymocytes. Upon TCR activation Y463 of CD5 is phosphorylated by Lck, and serves to recruit PI3-kinase, Vav, Ras, ERK and Rac. On the other hand, Fyn becomes activated and mediates c-Cbl phosphorylation. Phosphorylated c-Cbl can recognize this complex, and in this way can mediate its ubiquitin ligase function on CD5 and Vav. This model postulates that Y463 is a key residue relevant to the downregulation of TCR signaling mediated by CD5. TKB, tyrosine kinase-binding domain; 4H, four-helix bundle Ca^{2+} binding; EF, EF hand; L, short linker domain; RF: RING finger; UBA/LZ: ubiquitin-associated/leucine zipper like region. PR, Proline Rich region.

The results reported herein support a model (Fig. 4) by which sequences present in the carboxy-terminal region of hCD5, as opposed to the putative binding site in the pseudo-ITAM, appear to be relevant for c-Cbl phosphorylation, association and c-Cbl-dependent ubiquitylation of Vav. The absence of the pseudo ITAM in hCD5 did not interfere with c-Cbl phosphorylation in response to mCD3 and hCD5 co-crosslinking, but in contrast, this response was increased. Therefore, c-Cbl phosphorylation may not require physical association with hCD5 cytoplasmic tail, but rather, may indirectly associate with hCD5 through the interaction with other SH2-SH3 domain-containing molecules, such as PI3K, Vav and/or Lck, which could be recruited to hCD5 through Y463. In addition, our proposed model is in agreement with the recently described role of Fyn in the negative regulation of TCR signaling [7] and suggests that c-Cbl may also be recruited to hCD5 through a direct interaction with Fyn, which also required the carboxy-terminal region of CD5 to mediate its regulatory role. Finally, the pseudo ITAM region of hCD5 may play a crucial role in RasGAP mediated TCR downregulation, through a mechanism that involves the inactivation of Erk [21].

Acknowledgments

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