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REVIEW

The multiple faces of CD5

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Abstract

Since its discovery, over 30 years ago, CD5 has been used as a marker to identify T cells, B1-a cells, and B cell chronic lymphocytic leukemia cells. Throughout the years, many studies have described the functional relevance of CD5 as a modulator of T and B cell receptor signaling. However, it has not been until recent years that CD5 has emerged as a functional receptor in other areas of the immune system. Here, we review some of the most important aspects of CD5 as a modulator of TCR and BCR signaling, cell survival receptor both in T and B cells during health and disease, as well as the newly discovered roles of this receptor in thymocyte selection, T cell effector differentiation, and immune tolerance. CD5 was found to promote T cell survival by protecting autoreactive T cell from activation-induced cell death, to promote de novo induction of regulatory T cells in the periphery, to modulate Th17 and Th2 differentiation, and to modulate immune responses by modulating dendritic cell functions. CD5 is overexpressed in Tregs and Bregs, which are fundamental to maintain immune homeostasis. The newly established roles of CD5 in modulating different aspects of immune responses identify this receptor as an immune checkpoint modulator, and therefore it could be used as a target for immune intervention in different pathologies such as cancer, autoimmune diseases or infections.

KEYWORDS

apoptosis, B lymphocytes, signal transduction, T cell responses, T lymphocytes, thymus, tolerance/suppression/anergy

1 | INTRODUCTION

CD5 is a T cell, B1-a cell, and B-CLL receptor first described over 30 years ago that has been used as surface marker to identify both human and murine cell populations.[1,2] However, it was not until the mid-1980s and early 1990s that molecular and functional studies about the role of CD5 were performed.

This receptor was reported both as a positive and negative regulator of T cell receptor (TCR) signaling and as a negative regulator of BCR signaling (reviewed in Ref. 3). More recent studies have shown a major role of this receptor in regulating cell survival in these cells. Nevertheless, several interrogations about the role of CD5 in modulating these and other immune processes remained unanswered. Here, we review the most relevant findings that have provided new insights into CD5 biology and its functional relevance in the immune system.

2 | CD5 GENERAL REMARKS

CD5 (Leu-1) is a member of the SRCR family, which was initially described as a cell surface marker in human T cells and B-CLL cells using OKT1 antibodies.[2] Its expression was also reported in mouse T cells using anti-Lyt-1 antibodies and was shown mainly in thymocytes and mature T cells.[4] Later studies in humans showed that CD5 is also expressed in human fetal lymph nodes, specifically in a B cell population that appears to be different from the one generated in bone marrow.[5] In mice, it was shown that CD5 can define the B1-a cell subpopulation whereas in human B cells the expression of this receptor has also been observed in cord blood and peripheral B cells in

Abbreviations: AICD, activation induced cell death; AKT, protein kinase B; B-CLLB, B cell chronic lymphocytic leukemia; CD, cluster of differentiation; cDC, conventional DC; CK2, casein kinase 2; DAG, diacyl glycerol; DC, dendritic cell; DN, double negative; DP, double positive; EAE, experimental autoimmune encephalitis; gp, glycoprotein; GSK3, glycogen synthase kinase 3; IgVH, immunoglobulin variable heavy chain; mAb, monoclonal antibody; PC-PLC, phosphatidylcholine-specific phospholipase; pDC, plasmacytoid DC; pERK, phosphorylated ERK; PI3K, phosphatidylinositol 3 Kinase; PKC, protein kinase C; PMA, phorbol myristate acetate; pMHC, Mayor histocompatibility complex with associated peptide; RORγt, receptor tyrosine kinase-like orphan receptor γt; S, serine; SRCR, scavenger receptor cysteine rich; WT, wild type; Y, tyrosine

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children and also low CD5 levels have been observed in almost every B cell subset in adults.[6–8]

Recently, it has been reported that human cDC2 in tonsils, lymph nodes (LN), and blood as well as skin Langerhans and dermal DCs and blood pDCs can be further subdivided into CD5 positive and negative subpopulations, demonstrating for the first time that some human DCs express CD5.[9–11] In this context, it has been observed that CD5 is also expressed in DCs in mice (Burgueño-Bucio et al., unpublished data). The expression of CD5 in DCs might have some important consequences in the immune system; some of which will be later discussed.

3 | REGULATION OF CD5 EXPRESSION

To understand the functional relevance of CD5 expression on different immune cells, it is important to investigate how CD5 expression is regulated. It is known that CD5 levels differ between T cell subtypes and also B1-a cells. In T cells, levels of surface CD5 are differentially expressed as follows: thymic CD4+ > splenic CD4+ > thymic CD8+ > splenic CD8+ > thymic DP, with B1-a cells showing lower levels than DP thymocytes. In this context, the hierarchy of CD5 expression was shown to correlate with TCR-signaling strength at specific developmental checkpoints during T cell development.[12]

Despite human, murine, and avian CD5 genes were first cloned during the 1980s and 1990s,[13–15] it was not until the 2000s that modulation of CD5 expression was better understood. Molecular characterization of the murine CD5 gene showed that its promoter region contains three potential transcription factor-binding sites: the CCAAT, κE2, and E26 transformation specific (ETS), transcription site. Further characterization of these sites showed the ETS-binding region as an important site for the binding of the ETS-1 protein, and that deletion of this site results in an important reduction of CD5 expression. Additional to this region, two sites surrounding the ETS-binding domain, denominated CD5X and CD5Y, were identified as important modulators of CD5 full expression. It was shown that CD5Y differentially affects basal transcription of CD5.[16]

A different study unveiled the importance of the E47 transcription factor in regulating CD5 expression, by using T cell lines harboring CD5 mutations in the κE2 it was proven that E47 binds to this region and that its absence results in overactivation of the CD5 promoter. It was also shown that E47 expression is down modulated throughout thymic development, and this decrease directly correlates with an increment in CD5 expression. Finally, it was shown that in vivo injection of anti-CD3 in Rag-2 deficient mice results in induction of CD5 surface levels and reduction of E47 expression in DN3 thymocytes and that activation in vitro of DP cells by using anti-CD3 antibody results in activation of the CD5 promoter region.[17]

The molecular mechanisms involved in the regulation of CD5 expression are not yet fully characterized. However, it has been shown that TCR-signaling strength correlates with increased ETS expression[18] and downregulation of E47 corepressor,[17] which is in accordance with the differential CD5 expression on thymocyte subsets; i.e. CD4+ T cells expressing higher CD5 levels than CD8+ T cells require higher TCR signals for their selection than CD8+ T cells.

4 | IN THE SEARCH OF THE CD5 LIGAND

One of the most important questions about CD5 that remains unanswered is the identity of its endogenous ligands. Several approaches have been used to address this; however, up to date there has not been a conclusive answer for this question. Initially, the CD72 receptor, expressed in B cells, was identified as probable ligand for CD5.[19] Also, different studies showed the interaction of CD5 with different glycoproteins such as gp35-40, gp150, and gp200, which was suggestive of different CD5 ligands. Additionally, the IgVH framework region of immunoglobulins was also identified as a possible ligand.[20] More recently, a possible interaction of CD5 with itself has also been reported.[21]

CD5, as a scavenger receptor, can also bind glucans present in the wall of extracellular fungi and also to hepatitis C virus.[22,23] However, since all these works have been performed in vitro and despite all the efforts, different independent studies are required to corroborate these findings or demonstrate additional interactions of CD5 in vivo.

5 | MOLECULAR BASIS UNDERLYING CD5 SIGNALING

Some of the previous studies imply in some degree a functional role of CD5; however, understanding the mechanisms through which CD5 mediates its effects is a work in progress and required an initial awareness of the regions within this receptor responsible for of these effects. Initial experiments, by two different groups, showed the presence of a functional domain near the C-terminal region of human CD5, comprising two serines (S459, S461). In these works, it was demonstrated that casein kinase 2 (CK2) is required for the phosphorylation, both constitutive and after activation of CD5; it was also proven that the CK2β subunit binds to this region and is crucial for these effects.[24,25] Besides the importance of the CK2-binding domain of CD5, it was also clearly established by different studies that this receptor contains at least three tyrosines, as well as two threonines, susceptible to phosphorylation within the cytoplasmic region. To assess this matter, different works have studied the role of tyrosines 429, 441, and 463 of the human variant of CD5. Some of these experiments showed that CD5 can be phosphorylated by Lck or Fyn kinases in the tyrosines 429 and 463, after OKT3 or pervanadate stimulation, and that Lck activity is required for in vivo phosphorylation of these sites.[26,27] Concerning the role of the cytoplasmic threonines of CD5, one independent study showed the importance of these residues (T410 and T412) in regulating protein kinase C (PKC)-mediated diacyl glycerol (DAG) generation through activation of CD5.[28]

Although some of the previously described experiments suggested that CD5 is not only a cell surface marker but also a functionally relevant molecule, in the following sections we will discuss some of the most relevant findings that demonstrated the functionality of this receptor in different immune processes.

6 | THE ROLE OF CD5 IN MODULATING TCR SIGNALING

6.1 | Initial view of CD5 as a positive modulator of TCR signaling

The first experiments assessing the role of CD5 in T cell biology were performed during the 1980s. One of these studies showed that the use of an anti-CD5 antibody (Tp67 10.2) together with an anti-CD3 antibody in human peripheral blood mononuclear cells enhances proliferation of T cells compared to the anti-CD3 alone, suggesting that CD5 positively regulated TCR signaling.[29] Another report showed that anti-CD5 together with plate-bound OKT3 antibody is sufficient to increase proliferation of resting human T cells, whereas plate-bound OKT3 antibody by itself is not enough to observe this effect. It was also shown that CD5 stimulation enhanced IL-2R expression and IL-2 secretion in resting human T cells, when acting together with plate-bound OKT3.[30] Altogether, these initial findings set the tone for future experiments, proposing CD5 as a positive regulator/costimulator of TCR signals.

6.2 | CD5 as TCR independent signaling receptor

Although the results showing the costimulatory properties of CD5 in TCR signaling, several experiments addressed the ability of CD5 to generate TCR independent signaling. For example, the use of a different antibody directed against CD5 (TS 43 Ab) in the presence of monocytes-induced T cell proliferation, IL-2R expression, IL-2 secretion, and Ca^{2+} mobilization[31]; nevertheless, the need for monocytes in these experiments suggested that other molecules or cellular interactions may be involved in the observed effects, therefore making unclear whether CD5 signaling alone (without TCR signaling) could induce T cell responses. Furthermore, it was shown that the use of the Cris-1 monoclonal antibody alone, directed toward CD5, induced some intracellular signal events different from those previously observed by stimulation of the TCR pathway with anti-TCR or anti-CD3 antibodies. Using soluble anti-CD5 (Cris-1) in the presence of monocytes was enough to induce proliferation of resting T lymphoblasts; however, the events observed by using this antibody alone did not trigger the inositol phosphate metabolism or Ca^{2+} mobilization, thus suggesting a different pathway than that observed in canonical TCR signaling. Additionally, the use of Cris-1 mAb induced PKC activity through the induction of DAG. Finally, it was also demonstrated that stimulation with Cris-1 mAb induces phosphorylation of different proteins, some of them related to the TCR-signaling pathway, thus showing a cross-talk between CD5 and TCR-signaling pathways. These results proved for the first time that CD5 by itself is enough to induce intracellular signaling events in T cells; however, it is apparent that some of these events are different from those observed in canonical TCR-signaling pathway.[32]

Some studies showed that CD5 is able to induce TCR-dependent and independent production of DAG in Jurkat cells. Interestingly, both of the TCR-dependent and TCR-independent pathways seem to be different. The TCR-related pathway depends upon recruitment and activation of PC-PLC, whereas the TCR-independent pathway induces de novo synthesis of DAG through a non-PC-PLC pathway.[33,34] Furthermore, one of the most relevant findings in this study was showing a functional requirement of the distal region of CD5 for regulating the DAG production, thus suggesting that some residues near the C-terminal end (CK2–binding domain) are important for the biology of this receptor. However, further characterization of the regions within CD5 that have functional properties is a work in progress up to this date.

6.3 | Current view of CD5 as a negative regulator of TCR signaling

While some experiments showed the role of CD5 as a possible costimulator/positive regulator of TCR responses in peripheral T cells, the generation and characterization of CD5-deficient mice challenged this view. Tarakhovsky et al. in 1995 showed that thymocytes from the CD5-deficient mice are hyperresponsive to anti-CD3 stimulation. It was also shown that stimulation with anti-CD3 and PMA in both CD4+ and CD8+ developing T lymphocytes, but not in mature peripheral T cells, induces hyperproliferation of these cells. Additional experiments showed that anti-CD3 alone or together with anti-CD4 antibodies increases the Ca^{2+} mobilization and also induces hyperphosphorylation of proteins like PLCγ1, TCRζ chain, and Vav.[35] It was also reported that after CD5 stimulation there is an increase in phosphorylation of Fyn at its negative regulatory tyrosine, leading to the inhibition of ZAP-70.[36] Finally, experiments using different TCR-transgenic mice deficient for CD5 showed conclusively that CD5 can modulate thymic selection by "fine tuning" the TCR signal intensity.[12,37,38] These experiments, together with a report showing a possible interaction of CD5 with SHP-1 by using CD5-CD6 chimeras,[39] strongly suggested a role for CD5 as a negative regulator of TCR signals. The role of SHP-1 mediating CD5 negative regulation remains controversial; although a recent report has shown that this molecule is associated with CD5 and that this association is increased after TCR stimulation and depends on the phosphorylation of CD5 tyrosines, the analysis of SHP-1 deficient mice demonstrated that there are no differences in CD5 expression, in the phosphatase activity coimmunoprecipitated with CD5 or in the calcium mobilization after TCR/CD5 crossl-inking between SHP-1 deficient and sufficient cells. In this study, it was also shown that the deficiency of SHP-1 does not affect the inhibition of positive selection seen in H-Y TCR transgenic mice overexpressing CD5.[40] These suggest that CD5 is not a substrate of SHP-1 and is not involved in the negative regulation mediated by CD5. However, the association of SHP-1 to CD5 and the localization of CD5 in the immunological synapse may play a role in TCR-signaling regulation.

Additional studies, supporting the role of CD5 as a negative regulator of TCR signaling, have also been published. These works have shown the association of proteins related to the negative regulation of TCR-signaling pathway, like c-Cbl, RasGAP, or PI3K, to CD5 peptides comprising the 429–441 tyrosines or the 463 tyrosine.[41,42] Sequence analysis of the 429–441 region showed that this region resembles ITAM domains present in other receptors; however, different functional studies suggest it acts more like an ITIM domain than an ITAM domain (this will be discussed later). Recently, it has been

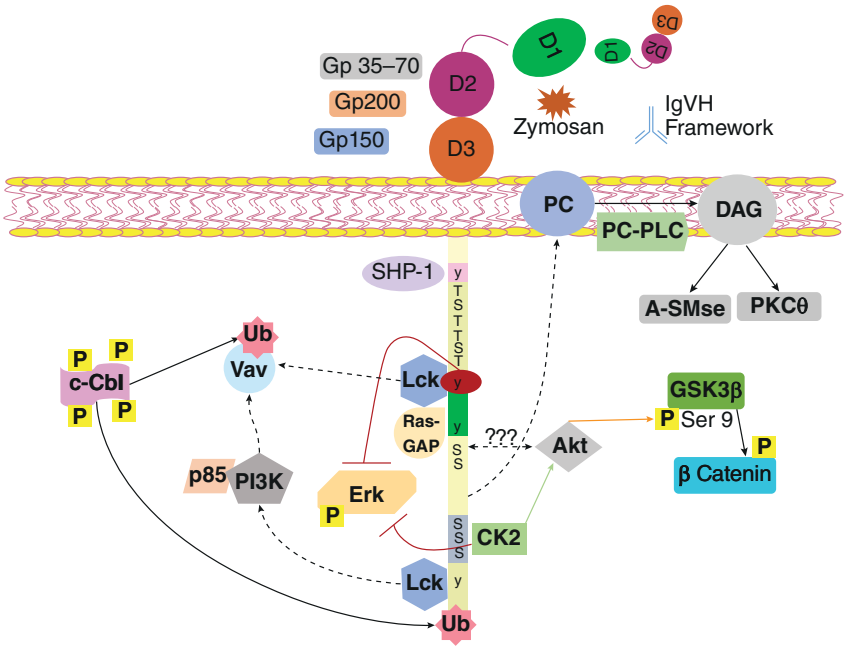


FIGURE 1 CD5 regulates different signaling pathways. To simplify, the postulated signaling pathways downstream of PC-PLC (phospha tydilcoline-dependent phospholipase) mediated activation of A-SMase (alpha sphingomyelinase) are not depicted. After activation through its extracellular domains, CD5 is able to modulate different signaling pathways. Through the binding of CK2, CD5 is able to activate AKT-mediated pathways and negatively regulate ERK phosphorylation. Additionally, Y429/441 residues are able to negatively regulate TCR signaling and survival through ERK inhibition. C-Cbl activation and degradation of Vav and CD5 itself has also been demonstrated to be regulated by the C-terminal region of this receptor. Finally, other signaling pathways have been proposed to be modulated through CD5, including phosphorylation of Vav and PI3K by Lck as well as RasGA- mediated downmodulation of TCR signaling

demonstrated that CD5 is an important scaffold for Cbl and Cbl-b ubiquitinylation following TCR engagement.[43] In this context, Roa et al. have shown the importance of the carboxy-terminal region of human CD5 in c-Cbl-mediated degradation of Vav, thus reinforcing the role of this receptor as a negative regulator of TCR signaling.[44] Some of the extracellular ligands and intracellular signaling molecules that interact with CD5 are exemplified in Fig. 1.

Most of the experiments showing the role of CD5 as a positive modulator of TCR responses were performed on peripheral T cells or mature T cell lines (Jurkat cells), whereas the majority of the initial experiments showing a role of CD5 as a negative regulator were mainly performed on immature T cells; these led to the proposal of a differential role of CD5 in modulating immature and mature T cell responses. A limited number of experiments regarding this matter have been performed. One report showed that cross-linking with anti-CD5 antibody in thymocytes, but not in splenocytes, increased the tyrosine kinase activity of CD5-associated CK2, suggesting that CD5 might work in a different way in developing and mature T cells.[45] Recently, a work by Axtell et al. showed that mature T cells in CD5-deficient mice as well as CD5-CK2 mutant mice hyperproliferate in a secondary response to CD3 cross-linking or myelin oligodendrocyte glycoprotein (MOG), these results clearly suggest that mature T cells responses are negatively regulated by CD5.[46] Despite these works, the question about a differential role of CD5 in thymocytes and mature T cells remains unanswered and the most accepted view of CD5 is that of a negative regulator of T cell receptor signaling.

7 | ROLE OF CD5 IN B CELL IMMUNOBIOLOGY

An important role of CD5 in the B cell biology was first suggested by the identification of CD5 on the surface of B cells in a B-CLL. Later, it was proven that CD5 is expressed not only on the surface of B-CLL cells but also in a subpopulation of B cells found in the human fetal bone marrow, spleen, and lymph nodes. Additional characterization of mouse lymphocytes showed the presence of CD5 in a subpopulation of B cells, denominated as B1 cells and now referred as B1-a cells (B1 cells are now subdivided in CD5- B1b cells and CD5+ B1a cells).

As we previously mentioned, CD5 expression patterns are not the same in human and mouse lymphocytes[47]; this raises a very important question: What drives the differences in CD5 expression of human versus mouse B cells? In this context, it has been demonstrated that an alternative Exon1 (E1B) for CD5 exists only in human B cells and that this E1B encodes a truncated CD5 protein that remains inside the cytoplasm. Activation of T cells induces the expression of the regular Exon 1 (E1A) only, whereas activation of B cells induces expression of both E1A and E1B. It has also been found that pre-B cells express mainly the E1B exon while B-CLL cells express mainly the E1A exon thus explaining why pre-B cells do not express CD5 whereas B-CLL cells do.[48]

Regarding the function of CD5 in B cells, the relationship between CD5 and the BCR was first described by Lankester et al. in 1994. By using B-CLL cells, it was shown that CD5 associates with the BCR-Igα complex and that ligation of the BCR complex induces tyrosine phosphorylation of CD5.[49] A different study performed in CD5-deficient B1 cells demonstrated the role of this receptor as a negative regulator of the BCR signaling; the use of anti-IgM antibodies to cross-link the BCR showed that the absence of CD5 enhances the proliferation of B cells. Further characterization of these CD5-deficient B1 cells also showed a decrease in apoptosis and a faster progression to the S phase of the cell cycle. Finally, it was also demonstrated that the presence of CD5 on the B1 cells inhibits NFκB translocation to the nucleus.[50] Although these findings clearly showed the role of CD5 as a negative regulator of BCR signals in B1 cells (both B1-a and B1-b cells), the specific role of CD5 in B1-a cells could not be assessed as CD5 is the marker used to identify these cells.

To determine the region and mechanisms through which CD5 regulates the BCR signals, different experiments have been performed.

Usage of a B cell lymphoma expressing a chimera, containing the extracellular portion of the low affinity IgG receptor (FcγRIIB) and the intracellular portion of the human CD5, has further confirmed the role of CD5 as a negative regulator of BCR signaling. In these experiments, it was shown that coligation of the BCR and the CD5-IgG chimera induces a reduction in Ca^{2+} mobilization and ERK 2 activation; interestingly, the effects observed appear to be independent of SHP-1 recruitment and associated with CD5.[51] Moreover, in this work it was also shown that a CD5-IgG receptor chimera lacking the region comprising tyrosines (429–441) is unable to negatively regulate the BCR-signaling pathway. Further studies showed that Y429, but not Y441, is required to inhibit the BCR responses.[52] These results strongly support the idea that the 429–441 region within the cytoplasmic acts as a functional ITIM (negative regulator) domain in B cells. Concerning the potential mechanisms of this negative regulation of BCR signals, it is worth mentioning that there is opposing evidence suggesting that a SHP-1 mechanism, mediated by Lyn activity is, at least partially, important for the CD5-mediated effects observed on the BCR signaling,[53] although this remains controversial.

The role of CD5+ B cells in cancer has also been controversial. CD5+ B cells have been proposed to possess a propensity for malignant transformation due to the self-renewing property they possess. Interestingly, regulatory B cells have been shown to express CD5. Recently, it has been reported that a subpopulation of intratumoral CD5+ B cells that do not express IL-6Rα is able to bind IL-6 through CD5 directly, leading to the activation of gp130 and Jak3 thus inducing the activation of STAT3 which, in a feed-forward loop, increases the expression of CD5. Using the B16 melanoma tumor model, this report also demonstrated that CD5+ but not CD5- B cells promoted tumor growth, indicating that CD5 might act as a modulator of tumor progression through direct binding to IL-6 triggering of signaling pathways such as the JAK-STAT pathway.[54] However, in contrast to IL-6R that can induce signaling via its soluble form, the soluble form of CD5 does not have this ability, although it can bind IL-6.[55]

Finally, it has also been shown that a CD5 single nucleotide polymorphism (SNP) (CD5V471A) seems to correlate with a worse outcome in B-CLL patients.[56] Although little is known about the role of this SNP in CD5-mediated functions, recent data have shown that B-CLL patients that bare an ancestral haplotype with a proline instead of a leucine at position 224 (P224L) and an alanine instead of a valine at position 471 (A471V) present better prognosis and longer lifespan, and the same has been reported in melanoma patients.[57,58] In this context, this SNP also appears to be biologically relevant in T cells, as suggested by a recent report showing that T lymphocytes carrying the ancestral 471 alanine have an enhanced proliferation and produce increased levels of IL-2 and IFN-γ when stimulated with anti-CD3 antibodies.[59]

8 | THE ROLE OF CD5 IN LYMPHOCYTE SURVIVAL

The role of CD5 in lymphocyte apoptosis/survival has been studied both in B and T cells. However, it remains to be fully explored if the activities of CD5 as a negative regulator of TCR and BCR signaling and as a modulator of cell survival are independent of each other or are somehow related. In this section, we will discuss some of the studies that have helped in the understanding of the role of this receptor as a cell survival modulator.

8.1 | Role of CD5 in thymocyte survival

The first experiments that suggested the role of CD5 in regulating T cell survival were performed in developing thymocytes. These experiments demonstrated that engagement of CD3 and CD5 using specific antibodies results in pERK induction; in this context, the role of pERK as an antiapoptotic molecule has long been known (reviewed in Ref. 60). Furthermore, these experiments also showed that cross-linking of CD3 and CD5 with monoclonal antibodies induces the expression of Bcl-2. Interestingly, this induction is ablated by the use of PD9805, a specific pERK inhibitor.[61]

By using CD5-deficient mice, it was demonstrated that thymi obtained from 12-week-old mice have reduced cellularity compared to those from WT mice, as a result of increased thymocyte apoptosis and an overactivation of Caspase 3. Interestingly, this increased cell death correlated with an impaired AKT phosphorylation of CD5-deficient thymocytes after CD3 and CD28 cross-linking, suggesting a major role of AKT in CD5-mediated protection from apoptosis of developing T cells.[62]

How CD5 modulates thymocytes survival has not been fully elucidated. However, it was recently reported that CD5-CK2 signaling pathway is in part responsible for the effect of this molecule in thymocyte survival. Using a gene-targeted mouse in which the CD5-CK2 binding domain was selectively ablated, Mier et al. determined the functional role of this domain in T cell survival during thymic development.[63] This experiment showed that the inability of CD5 to bind CK2 during thymic development results in an increased generation of CD4+ T cells at the expense of a reduced number of DP cells. This shift in the thymic subpopulations is the result of at least two combined effects: First, the absence of the CD5-CK2 binding region results in an increased apoptosis of DP cells, although it also affects CD4+ T cells and CD8+ T cells; and second, the absence of this domain increased pERK levels which results in altered negative and positive selection processes.[63] Interestingly, increased pERK levels did not correlate with increased TCR proximal signaling, thus suggesting that the CD5-CK2 binding domain, in addition to regulating thymocyte survival, modulates TCR signaling by an alternative noncanonical pathway (Fig. 2).

To determine whether the tyrosine residues within CD5 play a role in modulating T cell survival and AKT activity, Mier et al. created different CD5 point mutants and transfected them in EL4 thymoma cells. Analysis of four different mutants showed that mutation of the Y429 residue results in an increased apoptosis of these cells without any stimuli or after activation with anti-CD3 antibodies. One very interesting feature of this mutation is that the observed apoptosis is correlated with an increased phosphorylation in ERK 1/2; however, it does not seem to be related to pAKT. Although EL4 clones with containing the 441 and the 463 mutations also displayed increased baseline

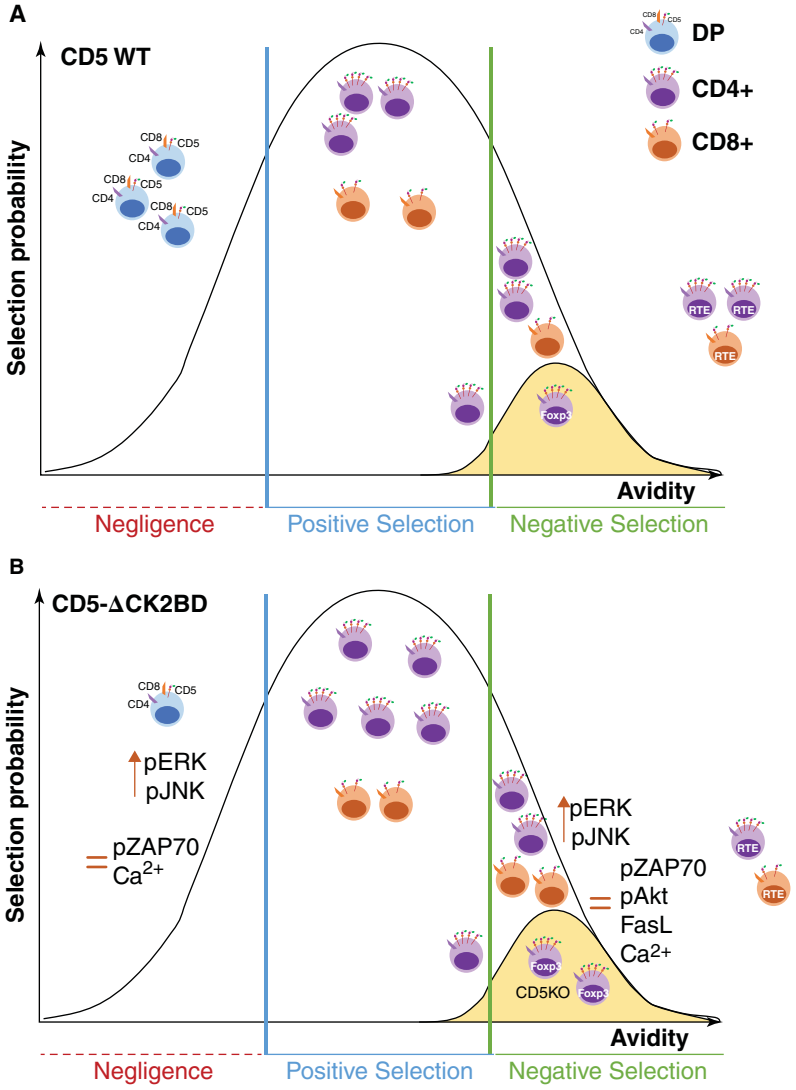


FIGURE 2 **Role of CD5 during thymocyte selection and central tolerance.** An adequate selection during thymocyte development is a crucial process to generate tolerance (central tolerance). The role of CD5-CK2 binding domain has been addressed using gene-targeted knock-in mice. (**A**) CD5 modulates thymocyte selection at the DP (with low to intermediate avidity) to CD4+/CD8+ single positive transition. High avidity of the TCR promotes negative selection as well as generation of Treg cells. (**B**) The absence of the CK2-binding region promotes an increased selection of CD4+ cells by affecting both selection signals and cell survival. The absence of this region also promotes a reduction in RTE (recent thymic emigrants) and a preferential death of CD8+ single positive thymocytes. The proteins affected by this region are pERK and pJNK, whereas TCR proximal signals like ZAP70 and Ca^{2+} mobilization remain unaffected. Treg selection is only affected in the complete absence of CD5, whereas the absence of the CD5-CK2 binding region does not affect this process, suggesting the involvement of other CD5 domains

apoptosis, none of these events seemed to be related to pERK or pAKT. Furthermore, the data obtained from these experiments clearly show a major role of the Y429 residue in modulating T cell survival, through an ERK-dependent pathway. Furthermore, Y429 domain appears to function as a pseudo-ITIM motif involved in TCR down modulation, similarly to previous findings in B cells.[64] However, the role of pAKT in CD5-mediated T cell survival remains controversial and more experiments need to be performed to conclusively address the participation of this molecule.

8.2 | Role of CD5 in mature T cell survival

Several studies have demonstrated the role of CD5 as a promoter of mature T cell survival. Using cytotoxic T cell clones, Friedlein et al. demonstrated that clones with low expression of CD5 are more susceptible to AICD than those with high expression of CD5. Moreover, they demonstrated that exogenous expression of CD5 in CD5-deficient JURKAT cells protects them from AICD after PMA activation. Interestingly, utilization of a blocking anti-CD5 antibody induces the expression of FasL in cytotoxic T cell clones, which correlates with increased apoptosis.[65] Furthermore, it was shown that upregulation of CD5 in CD4+ autoreactive T cells protects them from Fas-mediated cell death.[66]

Further experiments suggesting the role of CD5 as a prosurvival molecule were performed by using an EAE induction model. Interestingly, CD5-deficient mice immunized with MOG peptide showed a significant decrease in the clinical score of EAE compared to WT controls (Fig. 3B). These findings were unexpected since the main role of CD5 as a negative regulator predicted an enhanced disease due to hyperactivation of T cells. Interestingly, despite the increased activation of autoreactive T cells, the EAE score was reduced as a result of enhanced AICD of CD5-deficient T cells compared to CD5 sufficient cells. To further prove these findings, blocking of CD5 with a recombinant protein was performed showing similar results.[67] Altogether, these studies have definitely proven that CD5 is not only a negative regulator of TCR signaling but also a promoter of T cell survival. However, not many works have studied the domains through which CD5 mediates its effects and the molecular mechanisms involved.

Regarding this issue, we previously mentioned in this review that the carboxy terminal region of CD5 contains a CK2-binding region, comprising S458–S461 residues. CK2 is a serine/threonine kinase that has been shown to be a major regulator of apoptosis/survival

in different cell types (reviewed in Ref. 68). In this context, MOG immunization of CD5-deficient mice reconstituted with a mutant CD5 lacking the CD5-CK2 binding domain, showed that the CD5-CK2 binding domain is important for the regulation of AICD in T cells, since the absence of this domain results in a similar phenotype to that observed in CD5-deficient mice immunized with MOG. These data demonstrated an important role of this domain in regulating CD5-mediated T cell survival[46,69]; however, if CD5-CK2 signaling pathway regulates mature T cell survival in the same fashion as it does in thymocytes, it remains to be studied.

Nonetheless, the CK2-binding domain of CD5 is not the only region that may modulate T cell survival. Some experiments have shown that CD5 peptides comprising the tyrosines 429 and 441, or the 463 tyrosine are able to bind to the PI3K p85-SH2 domain,[41] although the Y463 peptide binds to this domain with threefold greater affinity. Binding of the PI3K protein to CD5 is of great importance because one of the most relevant signaling pathways related to cell survival and cell cycle is the PI3K-AKT pathway (reviewed in Ref. 70). Little has been studied about the PI3K pathway and its relationship with CD5 signaling. However, some experiments have shown that PI3K is activated in human T lymphocytes following the simultaneous engagement of CD28 and CD5 in the presence of PHA, although this activation does not lead to an increase in AKT activity, measured by phosphorylation of the H2B.[71] A relationship between CK2 and AKT has been previously reported[68,72]; this prompted to analyze the role of this CD5-CK2 binding domain in modulating AKT levels. Interestingly, a recent study analyzing the role of the CK2-binding domain of CD5 in Th17 generation has shown that the absence of the CK2-binding domain in peripheral T cells significantly reduces the phosphorylation of AKT in these cells after stimulation with anti-CD3 alone or together with anti-CD5 or in Th17-polarizing conditions. Further characterization of this effect showed a reduction in GSK3β, although a direct correlation between the lack of pAKT and apoptosis needs to be demonstrated in these cells.[69,73] However, analysis of developing thymocytes showed that pAKT (under basal conditions and after TCR stimulation) is not affected by the CD5-CK2 binding domain,[63] pointing out to other regions of CD5, previously mentioned, as the probable modulators of CD5-mediated AKT activity in T cells.

8.3 | Role of CD5 in B cell survival

Little is known about the role of CD5 in B cell survival. Most studies have been performed in "abnormal" B cells (B-CLL cells), which might not reflect its exact role in the biology of "normal" B cells. Up to this date, the underlying mechanisms through which CD5 controls lymphocyte survival are not yet fully understood.

In this context, it was demonstrated that CD5+ B cells produce higher amounts of IL-10 than CD5- B cells, and that the ectopic expression of CD5 in CD5- B cells induces the production of IL-10. In addition, blockage of IL-10 with a monoclonal antibody induces more apoptosis of a subset of IL-10 secreting B cells compared to control mice and CD5- B cells are more susceptible to apoptosis after BCR stimulation than CD5+ B cells.[74] These findings indirectly suggest a role of CD5 in B cell survival through regulation of IL-10 levels in homeostatic conditions.

Further support on the role of CD5 as a prosurvival molecule in B cells was obtained from experiments performed in B-CLL cells, and CD5 transfected Daudi cells showing that CD5 is hyperphosphorylated on tyrosine residues in these cells under basal conditions, thus implying that constitutive activation of CD5 might enhance B cell survival in disease conditions.[75] The mechanisms involved in CD5-mediated B cell survival have yet to be fully understood; however, several reports have shed light onto the potential signaling pathways involved. It was demonstrated that anti-CD5 cross-linking induces PKC activation in a subset of B-CLL cells, which correlated with the induction of the Bcl-2 prosurvival family member, Mcl-1.[76] Further analysis of the mechanisms involving CD5 as a prosurvival molecule in B cells has shown that Lyn-mediated SHP-1 recruitment of CD5 in some B-CLL cells may be responsible for their increased resistance to apoptosis.[77] Interestingly, and opposite to T cells, in B cells evidence exists that suggests a role of CD5 as a proapoptotic molecule. Characterization of the B1 population in CD5-deficient mice has shown that the absence of CD5 in these cells protects them from apoptosis, although these data are difficult to interpret as the presence of CD5 defines the B1-a subpopulation (but not the B1-b population), and the differences between these two populations are yet to be clearly defined.[50] Additional studies supporting the role of CD5 as an apoptosis inducer propose that ligation of CD5 in resting B cells but not in T cells induces apoptosis.[78] In this context, it has been shown that anti-CD5 treatment in B-CLL can induce apoptosis in a subgroup of patients.[79–81]

Altogether, these findings suggest that CD5 mainly acts as a prosurvival molecule in B and T cells, but under some experimental or disease conditions (such as B-CLL) it might work as a proapoptotic molecule.

9 | CD5 AS A REGULATOR OF T CELL ANERGY

The immune system has the ability to mount a functional response toward almost everything; this is mainly due to the broad repertoire of TCRs and BCRs. In order to avoid unwanted responses, the immune system has several mechanisms to avoid self/reactivity (immune tolerance), which include negative selection, anergy induction, activation-induced cell death, and the generation of regulatory T cell (Tregs).[82,83] Importantly, CD5 has been shown to participate in all these processes. In this section, we will discuss some of the most relevant findings regarding the immunomodulatory role of CD5.

The impact of CD5 in the thymic development, and therefore in negative selection, has already been discussed above. Regarding T cell anergy (Fig. 3A), it has been reported that chronic exposure of CD8+ T cells to their cognate antigen makes them anergic and this state correlates with increased CD5 expression.[84] In this context, it has been shown that CD8+ responses against a B16 melanoma established tumor are higher in CD5-deficient compared to CD5 sufficient mice, mainly due to a more activated phenotype and enhanced cytokine secretion by CD8+ T cells infiltrating the tumor. These results argue

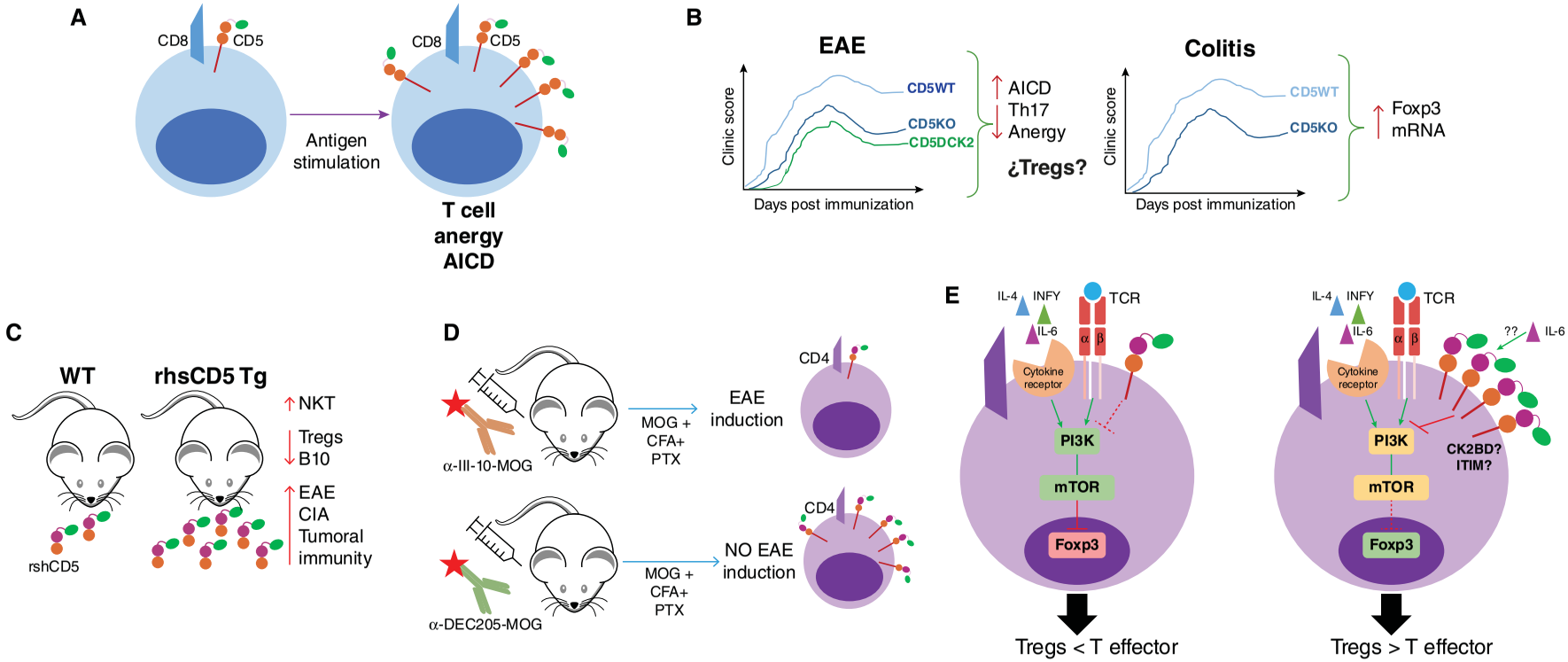


FIGURE 3 **Role of CD5 during peripheral tolerance.** CD5 has been implicated in several mechanisms of peripheral tolerance. (**A**) The expression of CD5 is increased when CD8 T cells are chronically exposed to their antigens rendering them anergic. (**B**) CD5 has been implicated in the outcome of various autoimmune models; in the absence of CD5, the disease has a delayed onset and less severity. This has been attributed in the case of EAE to an increased AICD and a reduction of T cell anergy and reduced Th17 cells. In the model of dextran-induced Colitis-induced colitis, an increased Foxp3 mRNA expression in the colonic Tregs was detected. (**C**) CD5 can be found as a membrane-bound glycoprotein or a soluble form; transgenic mice that overexpress this latter form induces an increase in NKT cells and a reduction of Tregs and B10 cells, which correlates with enhanced immune responses. (**D**) In a model of EAE, it has been reported that anti-DEC-205-MOG DC targeting is associated with no induction of EAE, due to an increased expression of CD5 in autoreactive T cells. It was later demonstrated that this increased CD5 expression favors the generation of Tregs by a mechanism that involves the inhibition of the PI3K/mTOR pathway activated by effector cytokines (IL-4, IL-6, IFN-γ), or alternatively could be the result of reduced TCR signaling mediated by CD5 overexpression

in favor of a role of CD5 as an inducer of T cell anergy; however, this increased antitumor response is transitory as CD5-deficient CD8+ T also showed increased AICD,[85] suggesting a shared role of CD5 in modulating anergy and cell survival. Additional studies have shown that antitumor activity of CD8+ T cells is inversely correlated with the expression of CD5 and that the expression of CD5 appears to correlate with the intensity of the interaction between pMHC and the TCR.[86] Interestingly, this study also demonstrated that tumor-infiltrating lymphocytes with low expression of pMHC decrease their expression of CD5 in order to lower their activation threshold. It has been reported that cytokine-primed CD8+ T cells present a rapid downmodulation of CD5 expression and that this leads to an increased proliferation, cytolytic activity, and IFN-γ production.[18] These results suggest that CD8+ T cells can modulate their CD5 expression in order to increase antigen responsiveness and overcome tumor escape.

Further studies addressing the role of specific domains within the CD5 cytoplasmic tail have shown that CD4+T cells from mice lacking the CD5-CK2 binding domain are more resistant to anergy.[69] This was evidenced in an EAE model where immunization with increasing doses of MOG, a model that mimics high dose tolerance, decreased disease severity in WT mice but not in CD5 or CD5-CK2 binding domain deficient mice (Fig. 3B), indicating that these mice did not reach tolerance. This was recapitulated in vitro, when CD4+ T cells from WT mice stimulated with anti-CD3 or MOG presented a reduced response and fewer cells entering cell cycle than CD4+ T cells from CD5 or CD5-CK2-deficient mice.

On the other hand, several reports indicate that CD5 levels regulate T cell sensitivity to antigenic responses in the periphery. In this context, response to an epitope of L. monocytogenes differs between two different T cell clones bearing distinct transgenic TCRs; LL056 transgenic T cells show high levels of CD5 expression, increased IL-2 production, and a more robust secondary response, whereas LLO118 T cells, which have lower levels of CD5, present a more robust primary response.[87,88] The enhanced T cell response of LL056 T cells correlated with an increased calcium mobilization at early times after stimulation compared to that observed in LLO118 cells. Moreover, CD5 appeared to play an important role in this Ca^{2+} mobilization, as the LL056 CD5-deficient T cells showed reduced Ca^{2+} responses compared to CD5 sufficient ones.[89]

10 | CD5 AS INDUCER OF IMMUNE REGULATION

As we previously mentioned, Tregs are a central part of the immune tolerance. Tregs are a subpopulation of CD4+ T cells that constitutively express CD25 and the transcription factor Foxp3. These cells exert a suppressive activity by cell–cell contact, metabolic disruption, cell lysis, and by the release of anti-inflammatory cytokines like IL-10, TGF-β, and IL-35.[90,91] Regulatory T cells can be generated in the thymus (tTregs) or in the periphery from naïve T cells (pTregs). The generation of Tregs requires several signaling pathways like the ones downstream the TCR, CD28, and the common gamma chain (γ_c chain). It has been described that the alterations in the function or expression of several signaling molecules like ZAP70, linker of activated T cells (LAT), PLCγ1, DAG, CD3ζ, PI3K, AKT, and mammalian target of rapamycin (mTOR) can affect the generation of Tregs (reviewed in Ref. 92). As we have mentioned above, some of these proteins have a known interaction with CD5; moreover, its role as a negative regulator of TCR signaling prompts the question as how does CD5 affect the generation of Tregs. In this context, Ordoñez et al. demonstrated that the absence of CD5 results in an increase of percentage and number of tTregs, which show a decreased susceptibility to apoptosis, as measured by Annexin V/ 7-AAD and caspase 3 activation. In contrast, naïve thymocytes from CD5-deficient mice showed enhanced cell death, which correlated with reduced levels of pAKT after stimulation with anti-CD3 + anti-CD28.[62] It has been reported that the PI3K/AKT/mTOR pathway inhibits Tregs generation, and therefore it is possible that the decreased Akt phosphorylation might favor tTreg generation in CD5-deficient mice.[93] Interestingly, CD5-deficient Treg cells showed increased suppressive activity over their CD5-sufficient counterparts,[62] in accordance with a previous report by Dasu et al., which demonstrated that CD5-deficient mice are less susceptible and have a delayed onset of dextran-induced colitis (Fig. 3B), which correlated with the increased expression of Foxp3 mRNA observed in the Tregs present in the colon of CD5-deficient mice.[94]

Additionally, it has been reported that a DC-targeting strategy toward DEC-205 coupled with MOG renders the treated mice resistant to the induction of EAE and results in an increased expression of CD5 in the antigen-specific T lymphocytes. CD5 overexpression made these T lymphocytes anergic to a second challenge with MOG, although they remained functional after anti-CD3 stimulation and thus this process was named "selective anergy."[95] A later work showed that the increased CD5 expression in "selectively anergic" T cells was followed by the conversion of these cells into Tregs. This report also showed that CD4+ CD25- T cells with high CD5 expression are more susceptible to become Tregs than CD4+ CD25- T cells with lower or null CD5 expression. Moreover, the high expression of CD5 rendered CD4+ CD25- T cells unable to respond to effector cytokines such as IL-4, IL-6, and IFN-γ, which induce the activation of the PI3K/AKT/mTOR pathway, known to inhibit Treg generation (Figs. 3D and E).[96] Treg induction in the periphery requires suboptimal TCR stimulation, which is provided in noninflammatory conditions or after chronic exposure to low amounts of antigen.[92] Interestingly, it has been reported that CD8+ $CD5^{hi}$ cells are more reactive to a TCR stimulus[97]; if this was true also for CD4+ T cells, one would expect a reduced Treg generation in CD4+ $CD5^{hi}$ cells, but instead there is an increase Treg generation, suggesting that CD4+ $CD5^{hi}$ T cells are less reactive to TCR stimulus. These discrepancies could be due to the differences in the biology of CD8 versus CD4 T cells or to intrinsic characteristics acquired by each cell subpopulation during thymic development.

Beside Tregs, there are other regulatory subpopulations including Bregs and NKTs. It has been reported that a soluble form of CD5, which comprises the three ectodomains, exists and is generated after TCR activation by proteolytic excision of membrane-bound CD5.[98] This soluble form can alter the percentage of some immunoregulatory populations like B10/Breg cells. This was shown in a transgenic mouse that overproduces the soluble form of human CD5, in which the

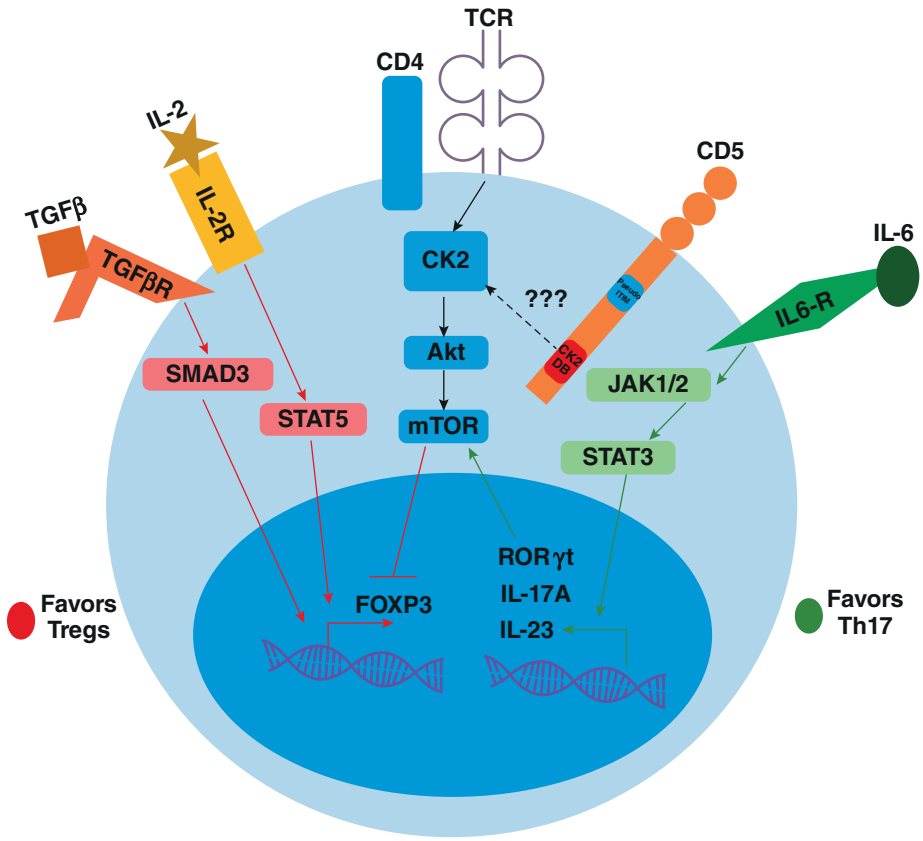


FIGURE 4 **CK2 regulates CD4 T cell differentiation**. After TCR activation, CK2 activity is upregulated which promotes the activation of the Akt/mTOR pathway. This pathway has been described to enhance Th17 and inhibit Treg differentiation. Additionally, CK2 enhances STAT3 activation in response to IL-6, possibly by an interaction with JAK, favoring Th17 differentiation. Pharmacological or genetic inhibition of CK2 does not enhance STAT3 activation, allowing the activation of STAT5 and Smad3 which favors Treg and inhibits Th17 generation. The mechanism by which association of CK2 to CD5 contributes to this effect is currently unknown

population of B10 cells was reduced in the spleen and peritoneal cavity, Tregs were reduced in LN, whereas the NKT population was increased in the spleen. All these effects are in agreement with the more aggressive EAE and collagen-induced arthritis autoimmune disease observed in these mice, and also correlated with a better antitumoral immunity (Fig. 3C).[99,100] In this context, as mentioned above, CD5 is important for the expression of IL-10 by B cells, and CD5 positive B cells are one of the major producers of IL-10 (Bregs). Therefore, one could suggest that in these transgenic mice, soluble CD5 might be blocking Bregs homeostasis.

11 | CD5 AS REGULATOR OF Th DIFFERENTIATION

CK2 has been reported to be important for the regulation of Th17/Treg differentiation. In this context, pharmacological inhibition, or genetic silencing of CK2 in CD4 T cells leads to increased Treg generation and decreased Th17 differentiation, possibly as a result of lower STAT3 phosphorylation and the inhibition of the PI3K/AKT/mTOR pathways (Fig. 4).[101–103] As CK2 is constitutively associated with CD5, the role of CD5 in T cell differentiation has been recently addressed. A report by Sestero et al. demonstrated that CD5 can regulate the differentiation of naïve T lymphocytes into different effector T cell subsets. Functional CD5-dependent CK2 signaling has been reported to be necessary for efficient differentiation of naïve CD4 T cells into the Th2 and Th17 lineage, but not to Th1. In a model of EAE, a reduction in the Th17 and IFN-γ+IL17+ subpopulations was found in CD5-CK2 deficient mice but not in WT or CD5-deficient mice, suggesting that the CD5-dependent CK2 pathway has a preferential role in the generation of IL-17 expressing cells or in the production of IL-17 per se.[69] Previous reports have shown that AKT enhances nuclear translocation of RORγt through the activation of mTORC1 and S6K2, and it also phosphorylates and inactivates GSK3β, reducing the IFNγR-induced STAT activation and NFκB activation.[104,105] In this context, as we previously mentioned, CD5 was shown to modulate AKT activity in thymic T cells; however, the modulation of AKT in peripheral T cells by the CK2-binding domain of CD5 was not proven until recently. This activation of AKT was shown to inhibit GSK3β, reduce STAT1 phosphorylation and T-bet levels, leading to a decrease in sensitivity to IFN-γ-mediated responses. This promoted an efficient nuclear translocation of RORγt, suggesting a mechanism by which CD5 may promote the differentiation of peripheral T cells toward a Th17 phenotype.[73] In concordance with these studies, it has been reported that costimulation of CD5 in human naïve CD4+ T cells increases Th17 differentiation and that these cells are better producers of IL-17 compared to those generated

by CD28 costimulation. This effect is due to a more prolonged activation of STAT3 and an increase in RORγt expression. Also, this effect appears to be dependent on the elevation of the IL-23R levels induced by CD5 costimulation and it can be overcome by a strong CD28 costimulation, suggesting that other cells than mature APCs are responsible for the generation of Th17 cells, or that APCs might express CD5 ligands that can induce Th17 differentiation.[106] Interestingly, these IL-23R induction does not seem to be dependent of the CD5-CK2 mediated signaling[69] suggesting alternative pathways through which CD5 can regulate IL-17/Th17 generation. Regarding the role of the CK2-binding domain of CD5 in Treg generation, it was reported that the absence of this domain did not affect tTreg generation[63]; however, unpublished data from our group (Burgueño-Bucio et al., in preparation) suggest that this domain does have an impact in iTreg generation.

12 | ROLE OF CD5 IN DENDRITIC CELL BIOLOGY

Lastly, there are very few studies regarding CD5 expression in other immune cell populations, besides T and B cells lymphocytes. Recently, it has been reported that human cDC2 in tonsils, LN, and blood can be subdivided based on their CD5 expression. Interestingly, those DCs with high CD5 expression showed increased migration toward CCL21, due to an augmented CCR7 expression, and induced higher T cell proliferation and IL-10, IL-17, IL-22, and IL-4 production.[9] Another study has demonstrated that skin Langerhans and dermal DCs can also be subdivided based on CD5 expression: CD5+ skin DCs are more potent at inducing CD8+ and CD4+ T cell proliferation and increased production of granzyme B, IFN-γ, and TNF-α in CD8+ T cells and IL-22 in CD4+ T cells.[10] Finally, it has also been proven that pDCs in blood can be further subdivided in CD5 positive and negative subpopulations and that CD5+ pDCs present a more mature phenotype, but that they do not produce great amounts of type I IFNs; nevertheless, they induce greater proliferation of B cells and induce plasma cell and Treg differentiation.[11] However, the functional relevance of CD5 in these different subpopulations of DCs needs to be further investigated. In this context, it has been observed that the majority of DC subpopulations in mice express CD5, although to a lesser extent than T cells and that the absence of CD5 has no effect on the generation of DCs, but it does affect their function, as CD5-deficient DCs appear to be more immunogenic (Burgueño-Bucio et al., unpublished data).

13 | CONCLUDING REMARKS

This review has shown that CD5 has a wide variety of functional activities in the immune system, although we still do not fully understand the complexity of this receptor. As CD5 modulates TCR and BCR signaling, Treg generation, anergy induction, and activation induced cell death, it can be considered an immune checkpoint inhibitor that, similarly to other negative costimulators such as PD-1, could be used to potentiate antitumoral T cell responses or promote immunity against infections and modulate autoimmune reactions.

Increased CD5 expression has been shown in anergized T cells after DC antigen targeting, whereas CD5 downregulation allows cytotoxic T cells to overcome tumor evasion and promotes cell death of autoreactive T cells and B-CLL cells. However, the use of CD5 as a target for immunotherapy still requires further understanding of the molecular mechanisms by which CD5 expression is regulated.

With the use of new technologies, that is generation of knock-in mice, it is now possible to dissect individual components within the CD5 cytoplasmic region, including the CK2-binding region and the ITIM domain, to further understand how CD5 modulates TCR signaling, lymphocyte survival, and immune tolerance. With this review, we aimed to boost the research into the molecular mechanisms underlying the immunomodulatory properties of CD5.

AUTHORSHIP

E.B.-B. and C.A.M.-A. contributed equally in the writing and reviewing of this article. G.S. reviewed and edited the article.

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DISCLOSURES

All the authors declare no conflict of interest

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