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Mini review

CD28 costimulatory signals in T lymphocyte activation: Emerging functions beyond a qualitative and quantitative support to TCR signalling

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ABSTRACT

CD28 is one of the most important co-stimulatory receptors necessary for full T lymphocyte activation. By binding its cognate ligands, B7.1/CD80 or B7.2/CD86, expressed on the surface of professional antigen presenting cells (APC), CD28 initiates several signalling cascades, which qualitatively and quantitatively support T cell receptor (TCR) signalling. More recent data evidenced that human CD28 can also act as a TCR-independent signalling unit, by delivering specific signals, which regulate the expression of pro-inflammatory cytokine/chemokines. Despite the enormous progresses made in identifying the mechanisms and molecules involved in CD28 signalling properties, much remains to be elucidated, especially in the light of the functional differences observed between human and mouse CD28. In this review we provide an overview of the current mechanisms and molecules through which CD28 support TCR signalling and highlight recent findings on the specific signalling motifs that regulate the unique pro-inflammatory activity of human CD28.

1. Introduction

Optimal T cell response to antigen is achieved following the recognition of peptide-major histocompatibility complex (MHC) by TCR (signal one) together with a subset of co-stimuli (signal two), generally provided by counter-receptors expressed on the surface of APCs. Since its discovery in early 1980s, CD28 has been considered the most prominent co-stimulatory molecule for optimal T cell clonal expansion, differentiation and effector functions. CD28 is a 44 kDa glycosylated, disulfide-linked homodimeric type I transmembrane protein expressed on the surface of 80% of human $CD4^+$ T cells, 50% of human $CD8^+$ T cells [1] and 100% of both murine $CD4^+$ and $CD8^+$ T cells [2]. CD28 binds to B7.1/CD80 and/or B7.2/CD86, expressed on the surface of activated APCs (i.e macrophages, dendritic cells, B lymphocytes), through a MYPPPY motif within its extracellular immunoglobulin (Ig)-V-like domain [3,4] and to B7-H2 through a region outside the MYPPPY motif [5].

The most discernable effects of CD28 ligation have been observed in concert with TCR stimulation. By delivering signals that complement TCR, in both qualitative and quantitative manners [6,7], CD28 promotes/enhances entry into and progression through the cell cycle, high levels of cytokines, cell survival and T cell differentiation [8]. More evidences have recently shown that CD28 may also deliver unique signals independent of TCR [9–13]. All the signalling properties of CD28 rely on specific and distinct motifs within its short cytoplasmic tails that initiate specific protein-protein interactions, thus activating downstream signalling cascades essential for CD28 costimulatory functions.

Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; APC, antigen presenting cell; Ig, immunoglobulin; IL, interleukin; IS, immunological synapse; PLCγ1, phospholipase Cγ1; LAT, linker for activation of T cells; Grb2, growth-factor receptor-bound protein; GADS, Grb2-related adaptor downstream of Shc; PKCθ, protein kinase C θ; ERK, extracellular signal regulated kinases; JNK, jun N-terminal kinases; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; AP-1, activator protein 1; UTR, 3'untranslated region; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3 kinase; Nck, noncatalytic region of tyrosine kinase; WASp, Wiskott Aldrich syndrome protein; Arp, actin related protein; PIP3, phosphatidylinositol 3,4,5-triphosphate; PIP2, phosphatidylinositol 4,5-biphosphate; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; IκB, inhibitor of NF-κB; IKK, IκB kinase; CARMA1, caspase recruitment domain membrane associated guanylate kinase protein-1; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; NIK, NF-κB-inducing kinase; STAT3, signal transducer and activator of transcription 3; MS, multiple sclerosis; RRMS, relapsing-remitting MS; Th, T helper; NOD, non-obese diabetic; EAE, experimental autoimmune encephalomyelitis; SH, Src homology; Itk, IL-2inducible kinase; PH, pleckstrin homology; PDK1, phosphoinositide-dependent protein kinase 1; PKB, protein kinase B; CD28SAbs, CD28 superagonistic antibodies.

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This review illustrates the most relevant functional roles of CD28 in T cell activation and highlights the complex signalling motifs and mechanisms that define its TCR-dependent and TCR-independent functions. Moreover, some controversial data and unresolved functional roles of human and mouse CD28 are also discussed.

2. CD28 support to TCR-signalling

In the absence of CD28 signals, naïve T lymphocytes fail to activate and fall into a state of profound unresponsiveness, known as anergy, which is characterized by the inability to produce interleukin (IL)-2 and/or to proliferate [14,15]. On the contrary, CD28 engagement by either agonistic antibodies or its natural ligands B7.1/B7.2 lowers the T cell activation threshold [16] and leads to the augmentation of TCR signalling events necessary for efficient cytokine production (via augmented transcriptional activity and messenger RNA stabilization), cell cycle progression, survival, regulation of metabolism and T cell responses. This occurs as CD28 is a crucial player for immunological synapse (IS) organization, where it enhances close contact between T cells and APCs [17]. These events likely rely on CD28 "adhesion effects", which are strongly dependent on CD28 capability to trigger actin cytoskeleton rearrangement events, which are necessary for the recruitment and the organization of molecular signalling complexes [18]. Particularly relevant for CD28-mediated costimulation of TCR signalling is the rearrangement of membrane lipid rafts [19], which generates a dynamic platform at the IS where many signalling proteins are concentrated and protected from phosphatases [20]. In this way, CD28 is able to act as a general amplifier of early TCR signalling pathways, such as tyrosine phosphorylation events, phospholipase Cγ1 (PLCγ1) activation and Ca^{2+} response [17,19,21].

At a biochemical level, CD28 engagement leads to the recruitment and activation of several molecular adaptors and effector proteins that are essential for the optimal activation of the biochemical events triggered by TCR (Fig. 1) [8]. Briefly, one of the earliest events initiated by TCR recognition of peptide-MHC complexes is the tyrosine phoshorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) of CD3 and ζ chains by Src family tyrosine kinases p56lck and p59fyn. Tyrosine phosphorylated ITAMs bind the Syk family tyrosine kinase Zap-70 that following activation by p56lck and/or p59fyn phosphorylates several important cellular proteins, thus inducing the activation of downstream signalling pathways. One critical substrate of ZAP-70 is the linker for activation of T cells (LAT) that following phosphorylation of specific tyrosine residues binds and recruits to the membrane PLC-γ1 and the growth-factor receptor-bound protein (Grb2) [22].

PLCγ1 idrolyzes phosphatidylinositol 4,5-biphosphate (PIP2) into inositol-1,4,5-trisphosphate (IP3) and diacylglicerol (DAG). Soluble IP3 triggers intracellular Ca^{2+} mobilization, thus leading to the activation of calcineurin and nuclear factor of activated T cells (NF-AT) [23]. Full PLCγ1 activation strictly depends on CD28 signalling, as demonstrated by experiments in which TCR ligation in absence of costimulation results in a strong reduction of both PLCγ1 phosphorylation and release of endoplasmic reticulum Ca^{2+} into the cytoplasm [17]. The membrane lipid DAG activates the protein kinase C (PKC)θ and nuclear factor-κB (NF-κB) [24]. CD28 stimulation is essential to trigger the activation of the NF-κB pathway by favouring the recruitment of PKCθ to the IS [25,26].

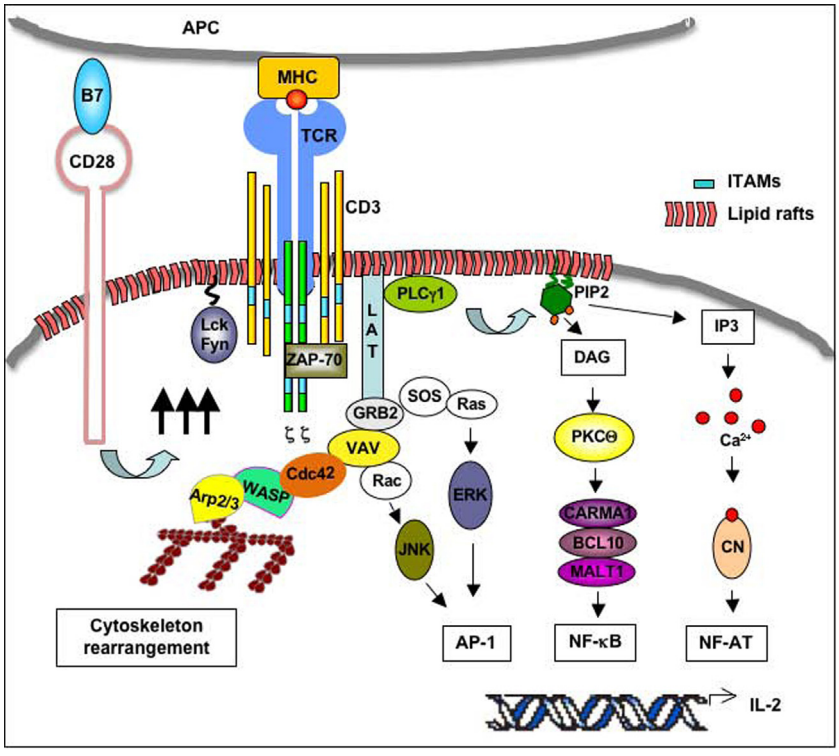

Fig. 1. CD28 amplifies early and downstream signalling pathways triggered by TCR. Upon TCR recognition of peptide-MHC complexes presented on the surface of APCs, Lck and Fyn phoshporylate CD3 and ζ chains, which bind ZAP-70. ZAP-70 phosphorylates LAT that in turn binds: PLC-γ1 that induces the activation of Ca^{2+}/Calcineurin (CN) and NF-AT as well as PKCθ/CARMA1/Bcl10/MALT1 and NF-κB; Grb2 that, by bringing SoS and Vav1, activates Ras/ERK and Rac-1/JNK, respectively, thus inducing AP-1; Grb2/Vav1 also activates Cdc42/WASP/Arp2/3 and actin cytoskeleton rearrangements necessary for the mobilization of membrane lipid rafts. NF-AT, NF-κB and AP-1 cooperate to transactivate the IL-2 gene promoter.

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Grb2 recruitment to LAT leads to the activation of the mitogen-activated protein kinase (MAPK) cascades. Indeed, Grb2 recruits Son of Seveless (SoS), a guanine nucleotide exchange factor for $p21^{Ras}$, that in turn activates the extracellular signal regulated kinases (ERK) pathway. Grb2 has been also implicated in the recruitment of Vav1, a guanine nucleotide exchange factor for small G proteins of the Rho family, Rac1 and Cdc42, and in the activation of Jun N terminal kinases (JNK). JNK and ERK cooperate to induce the transcriptional activation of AP-1 [27]. CD28 engagement by B7 cooperates with TCR for achieving the optimal activation of both ERK and JNK [21,28,29].

At a functional level, the most relevant outcomes of CD28 costimulation are the increase of T cell proliferation and cytokine production. CD28 strongly upregulates the expression of D cyclins by downregulating $p27^{kip1}$ [30,31]. Moreover, during the G_0–G_1 transition, CD28 is also able to upregulate the biosynthesis of macromolecules and to increase energy metabolism by activating the mammalian target of rapamycin (mTOR), thus inducing the expression of genes involved in translation initiation, synthesis of ribosomal RNA and cell division [32].

In T cells, optimal induction of cell proliferation following the antigen encounter depends on the IL-2/IL-2 receptor system [33]. Notably, CD28 signals enhance IL-2 expression and secretion at both transcriptional and post-transcriptional levels [34,35]. The IL-2 promoter is, indeed, specifically regulated by NF-AT, NF-κB and AP-1 transcription factors that, as described above, are all amplified by CD28 (Fig. 1) [8]. In addition to the classical NF-AT, NF-κB and AP-1 binding sites, the IL-2 promoter contains a specific enhancer region, highly conserved between human and mouse, known as CD28-responsive element (CD28RE). CD28RE is a specific target sequence for CD28-regulated nuclear binding complex that together with adjacent NF-IL-2B AP-1 sites, is part of a composite element, termed RE/AP [36,37]. This regulative unit controls CD28 responsiveness in the IL-2 promoter and results in a site for signal integration, thus mutations of RE/AP strongly impair the transcriptional increase of IL-2 induced by CD28 costimulation. Moreover, the identification of specific NF-κB binding sites within the CD28RE of IL-2 promoter, highlighted the relevant role of NF-κB in CD28 costimulation [37].

Finally, CD28 controls the level of IL-2 secretion by means of post-transcriptional mechanisms that involve the enhancement of both export and stability of IL-2 mRNA [35,38,39]. mRNA stability is largely controlled by AU-rich elements contained within the 3′ untranslated region (UTR) that affects mRNA degradation. In resting cells, AU-binding proteins, such as tristetraprolin, bind the 3′UTR of IL-2 mRNA and induce IL-2 mRNA degradation [40]. CD28 costimulation favours TCR-mediated nuclear export of NF90 that by binding the AU-rich elements increases IL-2 mRNA stability [38,39].

3. CD28 autonomous signalling: from cytoskeleton to NF-κB activation

Despite the relevance of CD28 in enhancing TCR-mediated activating signalling, CD28 is also able to act as a unique signalling receptor and to deliver TCR-independent autonomous signals [10,12,13], which finally lead to the regulation of both T cell survival [11,41] and cytokine production [9,42].

The unique nature of CD28 signalling strictly depends on its capability to promote cytoskeleton rearrangement events by recruiting Vav1 and filamin-A [43–46].

Vav1 is a critical guanine nucleotide exchange factor for Rac1 and Cdc42 and is required for CD28-dependent signals and actin nucleation [6,42,47,48]. Vav-1 is strongly tyrosine phosphorylated and activated by CD28 [47], binds CD28 following stimulation [43,49,50], thus bringing to the membrane the associated noncatalytic region of tyrosine kinase (Nck) β [43,51], a critical adaptor that promotes N-Wiskott Aldrich syndrome protein (WASp)/actin related protein (Arp) 2/3 complex localization and actin polymerization [52]. The Arp2/3 complex in turn cooperates with filamins in establishing cortical actin architecture [53].

Filamins are large cytoplasmic proteins that crosslink cortical actin into a dynamic three-dimensional structure and, by interacting with more than 70 proteins involved in cell signalling [54], may represent versatile signalling scaffolds. Filamin-A is predominantly expressed in the immune system and participates in T cell activation [46,55]. CD28 recruits filamin-A to the membrane, where filamin-A cooperates with Vav1 to integrate signalling pathways resulting in actin polymerization, lipid raft mobilization and T cell activation [44,46].

The dynamic and organization of actin cytoskeleton is also tightly regulated by membrane phosphoinositides, which may directly interact with key actin binding proteins, thus controlling the selective localization of scaffolding molecules linking the actin cytoskeleton to the plasma membrane [56]. Among the phosphoinositides, the best regulators of the actin cytoskeleton are phosphatidylinositol 3,4,5-triphosphate (PIP3) and phosphatidylinositol 4,5-biphosphate (PIP2), which are mainly generated by the activity of PI3K and phosphatidylinositol 4-phosphate 5-kinases (PIP5K), respectively [57]. CD28 recruits both class 1A PI3K and PIP5Kα and β, which in turn cooperate to generate the PIP2 and PIP3 levels necessary for filamin-A and Vav1 recruitments and activation as well as for promoting actin polymerization and CD28 signalling functions in human T lymphocytes [43,58,59].

The efforts made in an attempt to characterize the signalling pathways activated by CD28 in a TCR-independent manner, led to the identification of NF-κB as the most relevant CD28 biochemical target [42,60]. Since the first discovery of NF-κB binding sites within the CD28RE in the IL-2 promoter [37], a lot of efforts and progresses have been made to identify the mechanisms and molecules coupling CD28 to NF-κB activation.

3.1. NF-κB activation and CD28 pro-inflammatory functions

NF-κB family consists of five members that form homo and heterodimeric complexes including NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (p65), RelB and c-Rel. RelA, c-Rel and RelB contain transactivation domains and form transcriptionally active heterodimers in association with p50 and p52. Inhibitory proteins belonging to the inhibitor of NF-κB (IκB) family, which include IκBα, IκBβ, and IκBε regulate NF-κB activity. A protein kinase complex containing two serine kinases, IκB kinase (IKK)α and IKKβ and a third subunit, IKKγ/NEMO, with regulatory functions, phosphorylates IκBs, thus leading to their proteolytic degradation and release of NF-κB into the nucleus [61]. Two pathways have been described for NF-κB activation. The canonical pathway activates the tripartite IKKα/γ/β complex thus leading to phosphorylation-dependent IκB degradation and activation of RelA/p50 or c-Rel/p50 dimers. The non-canonical pathway activates IKKα homodimers, thus leading to the processing of NF-κB2 and the release of p52-containing heterodimers [62]. When co-engaged with TCR, CD28 contributes to the activation of the canonical pathway by recruiting PKCθ [25,26] and the ternary complex caspase recruitment domain membrane associated guanylate kinase protein 1 (CARMA1), Bcl10 and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) that links TCR to the IKK complex [63–65]. MEKK1 has been also involved in the activation of the IKK complex by CD28 [66,67]. The main functional effect of CD28 contribution to TCR-induced NF-κB activation is the increase of IL-2 transcription [68–70].

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Conversely, the stimulation of CD28 in the absence of TCR recruits and activates IKKα and a non-canonical NF-κB2-like cascade leading to the nuclear translocation and activation of RelA/p52 dimers [11,45]. Vav-1 and filamin-A cooperates with CD28 to induce the activation of this non-canonical NF-κB2-like cascade by binding and recruiting to the membrane IKKα [45] and the IKKα activator NF-κB-inducing kinase (NIK) [44], respectively.

CD28 unique signalling to NF-κB converges to the selective regulation of the expression of several genes, including anti- and pro-apoptotic gene of Bcl-2 family [41], the LTR of HIV-1 virus [71] and pro-inflammatory cytokine/chemokine [11]. The up-regulation of pro-inflammatory cytokines and chemokines by CD28 is particularly relevant in the context of inflammatory diseases, such as Multiple Sclerosis (MS), where we have recently observed that CD28 stimulation induces the selective up-regulation of IL-6, IL-21 and IL-17A expression in human primary T lymphocytes [9]. These cytokines are related to the T helper (Th) 17 cell phenotype [72] and have been found at higher levels in relapsing-remitting MS (RRMS) patients during exacerbations or undergoing a relapse [73,74]. The use of specific inhibitory drugs evidenced that the up-regulation of pro-inflammatory cytokines was dependent on CD28-associated class 1A PI3K activation [9,59]. The PI3K pathway plays a central role in regulating inflammation and its dysfunctions could be linked to the development of autoimmunity [75]. PI3K has been also involved in activating mTOR pathways, which govern Th17 differentiation [76,77]. All these data strongly suggest that CD28 may modulate the metabolic processes, which regulate specific pro-inflammatory T cell responses and the amplification of Th17 cells in inflammatory/autoimmune diseases. For instance, several mouse models of human inflammatory/autoimmune diseases, such as autoimmune diabetes in Non-obese diabetic (NOD) mice [78], MS in experimental autoimmune encephalomyelitis (EAE) mice [79] or systemic autoimmune disorders [80,81] have evidenced the relevance of CD28 costimulatory signals.

4. CD28 signalling motifs

CD28 signalling properties derives from tyrosine and proline-based motifs within its small cytoplasmic domain (41 aa), which bind the Src homology (SH) 2 and SH3 domain of intracellular signalling molecules. It contains a N-terminal YMNM motif that following phosphorylation binds the SH2 domain of the p85 subunit of class 1A PI3K and the adaptor proteins Grb2 and Grb2-related adaptor downstream of Shc (GADS) [8,82]. Downstream of the YMNM motif, CD28 has two proline-rich regions, the N-terminal PRRP that binds the SH3 domain of the IL-2 inducible kinase (Itk) [83], and a C-terminal motif PYAP that binds Lck [84,85], Grb2 [86], filamin-A and associated NIK [44,46], Vav1 and associated PIP5Kα and β [43,58].

4.1. The CD28-YMNM motif and PI3K-dependent functional properties

Engagement of CD28 by either agonistic antibodies or its natural ligands induces the tyrosine phosphorylation of YMNM, likely mediated by p56lck and p59fyn [87,88], that in turn binds the SH2 domain of p85 subunit of class 1A PI3K [89–92]. Class 1A PI3K phosphorylates PIP2 and generates PIP3 lipids that bind the pleckstrin homology (PH) domains of important signalling intermediates, such as phosphoinositide-dependent protein kinase 1 (PDK1), protein kinase B (PKB/AKT) and Vav1 [93]. PDK1 association with CARMA1 leads to the membrane recruitment of PKCθ [94] that is activated by PDK1 through phosphorylation on threonine 538 [26]. PKCθ co-segregates with CD28 to a

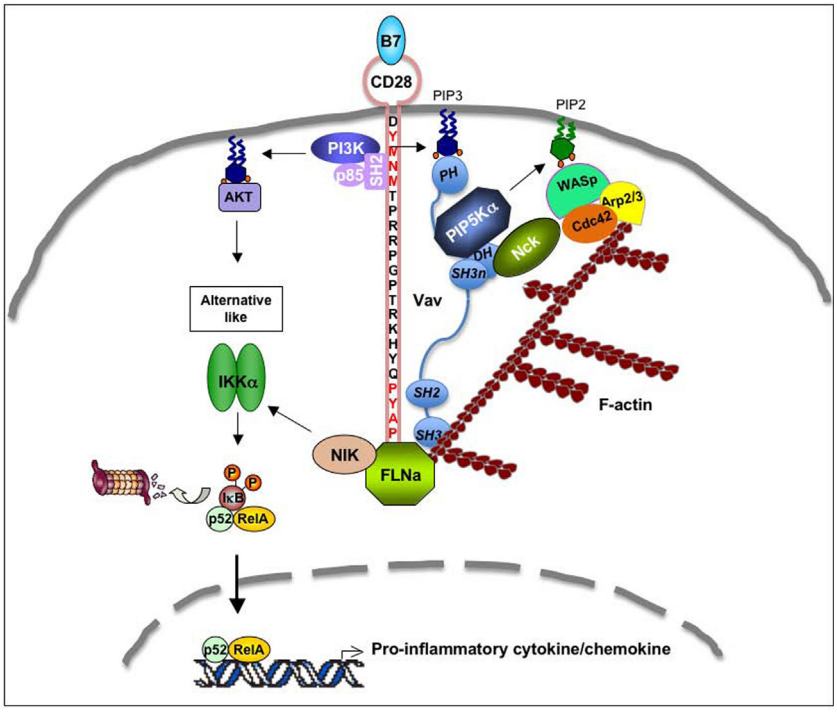

Fig. 2. Schematic model of human CD28-mediated activation of actin cytoskeleton rearrangement, non-canonical NF-κB pathway and pro-inflammatory cytokine/chemokine gene expression. Following engagement of human CD28 by B7, tyrosine phosphorylated YMNM binds the SH2 domain of p85 regulatory subunit of class 1A PI3K and tyrosine phosphorylated C-terminal YAPP binds Vav1 and associated PIP5Kα and Nck. PIP5Kα generates PIP2 that favours the recruitment of WASP/Cdc42/Arp2/3 complexes and Vav1/Nck complexes induce actin cytoskeleton reorganization and the recruitment of the actin binding protein filamin-A (FLNa) and associated NIK. Class 1A PI3K phosphorylates PIP2 and generates PIP3 that favours the recruitment and activation of Akt. Akt cooperates with NIK to activate IKKα and non-canonical p52/RelA NF-κB pathway, thus leading to the transcription of pro-inflammatory cytokine/chemokine genes.

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spatially unique subregion within the IS [95], where it favours the activation of CARMA1/Bcl10/MALT1 complex and IKKs [65]. PDK1 recruitment to the membrane also leads to the phosphorylation of Akt on threonine 308 [96,97], thereby favouring its activation. Once activated Akt cooperates with PKCθ in stimulating the NF-κB cascade [98]. For instance, Akt promotes TCR/CD28-mediated phosphorylation of Bcl10 and binding to CARMA1 [99]. In addition to cooperate with CD28 and PKCθ in activating the canonical NF-κB pathway [100,101], Akt synergizes with CD28 to phosphorylate Cot/TPL2 at serine 400 [102], thus leading to NIK-dependent activation of IKKα [103–105].

At a functional level, PI3K-dependent activation of Akt has been found to regulate the expression of survival [100,106,107], IL-2 and inflammatory genes [9,42]. The PI3K/Akt pathway enhances IL-2 secretion by inhibiting the nuclear export of NF-AT via glycogen synthase kinase 3 phosphorylation [108] and by favouring glucose metabolism required for DNA, RNA and protein synthesis [32]. More recent data evidenced a crucial role of PI3K/Akt pathway in favouring the phosphorylation of IL-6/IL21-associated signal transducer and activator of transcription 3 (STAT3) and IL-17A gene expression [76]. These results together with our data that CD28-mediated up-regulation of IL-6, IL-21 and IL-17A in MS patients is dependent on PI3K activation [9], also suggest a role of PI3K/Akt pathway in regulating CD28 unique pro-inflammatory functions (Fig. 2).

Importantly, recent data from knocking mice evidenced that mutations of the proximal YMNM motif and abrogation of PI3K binding has no detectable effects in vivo [109], thus suggesting the existence of differences between mice and human CD28 signalling properties (see below).

4.2. CD28 and the C-terminal proline rich motifs

In addition to the YMNM motif, CD28 intracytoplasmic tail contains two proline-rich motifs: a N-terminal P^{196}RRP (numbering in human CD28) that binds the SH3 domain Itk [83,110] and a C-terminal proline-P^{208}YAP (numbering in human CD28; P^{187}YAP in mouse) that recruits several important signalling proteins. While the function of P^{196}RRP and Itk in CD28 costimulation is still unclear [111–113], the PYAP motif is undoubtedly a key regulator of CD28 signalling functions. C-terminal PYAP motif was originally reported to bind the SH3 domain Lck [84], thus sustaining its kinase activity and favouring lipid raft recruitment to the IS [85]. More recent studies revealed an important contribution of the C-terminal PYAP motif in regulating the localization of CD28 at IS [114]. Yokosuka et al. evidenced that the PYAP motif regulates the recruitment of PKCθ to the IS and its colocalization with CD28 [95]. Tavano et al. found that this motif is also important for filamin-A recruitment [46], thus suggesting a critical role of PYAP motif in regulating both canonical [95] and non-canonical NF-κB pathways [44]. Indeed, Watanabe et al. reported that the substitution of the two proline residues in the C-terminal PYAP motif of murine CD28 strongly reduces NF-κB transcriptional activity [115]. Furthermore, we found that mutations of tyrosine residues within human C-terminal YQPYAPP also result in impaired IKKα activation and NF-κB-dependent transcription of target genes, thus suggesting the involvement of SH2 binding motifs [43,44,46,71].

Vav1 is the critical regulator that couples the C-terminal PYAP of CD28 to the activation of downstream signalling pathways. Vav1 recruitment to the C-terminal PYAP of CD28 is, indeed, essential for CD28 autonomous signalling leading to NF-AT, NF-κB and IL-2/IL-4 transcription [12,45,116]. On the basis of the ability of the adapter molecules Grb2 to interact with both CD28 [86,117] and Vav1 [118,119], Grb2 binding to CD28 has been suggested as the mechanism by which CD28 recruits Vav1 [49,50]. However, when we looked at Grb2 recruitment to CD28 in human primary $CD4^+$ T cells, we did not find any association between CD28 and Grb2 [43]. Conversely, we found that, in human primary $CD4^+$ T cells stimulated by B7, CD28 associates with Vav1 through its C-terminal PYAPP in an SH2-dependent manner [43]. Moreover, we also demonstrated that the cooperative signalling function of Vav1 relies on its ability to promote the actin polymerization events required for the efficient recruitment and activation of essential signalling complexes to CD28. For instance, by binding the C-terminal YAPP motif of human CD28, Vav1 favours the recruitment of associated Nckβ [51] and PIP5Kα/β [43,58]. PIP5Ks in turn synthesize PIP2 lipids that synergize with Nckβ in promoting N-WASp-dependent actin rearrangements [120] and the recruitment of filamin-A and associated NIK [44,46], thus inducing NF-κB-dependent pro-survival and pro-inflammatory gene expressions (Fig. 2).

5. Signalling differences between human and mouse CD28

Until 2006, the signalling properties of CD28 between rodent (mouse and rat) and human were considered rather similar. For

Table 1
CD28 superagonistic antibodies in experimental models.

Antibody	Species	Functional properties
JJ316	Rat	Stimulating in the absence of TCR No pro-inflammatory effects Expand regulatory T cells Prevents EAE
D665	Mouse	Stimulating in the absence of TCR No pro-inflammatory effects Expand regulatory T cells Protect from EAE and GVHD
5.11A1	Humanized Mouse	Stimulating in the absence of TCR No pro-inflammatory functions Increases thymic cellularity Induces peripheral T cell depletion
TGN412	Human	Stimulating in the absence of TCR Pro-inflammatory functions Induces severe systemic inflammatory cytokines release
	Monkey	Stimulating in the absence of TCR No pro-inflammatory effects Does not induce severe systemic inflammatory cytokines release

EAE, experimental autoimmune encephalomyelitis; GVHD, graft-versus-host-disease.

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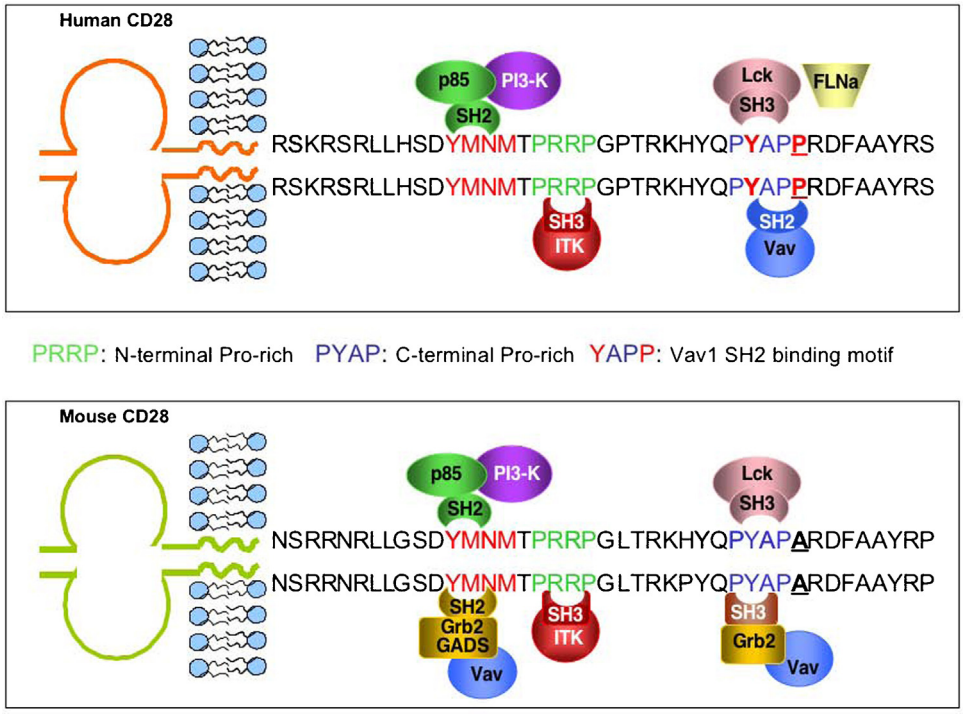

Fig. 3. The sequence and signalling molecules recruited to human and mouse CD28 cytoplasmic motifs. The sequence of SH3 and SH2 binding motifs within human and mouse cytoplasmic tails of CD28 is shown. The YMNM motif binds the SH2 of p85 subunit of class 1A PI3K in both human and mouse as well as Grb2/Vav1 complexes in mouse. The N-terminal PRRP motif binds the SH3 domain of Itk in both human and mouse. The C-terminal PYAP motif binds the SH3 domain of Lck in both human and mouse, Grb2 in mouse, and filamin-A (FLNa) in human. Tyrosine phosphorylated C-terminal YAPP of human CD28 is a consensus sequence for the SH2 domain of Vav1 and binds Vav1.

instance, in both human and mouse, CD28 cooperates with TCR in activating PKCθ, PLCγ1, ERK and JNK kinases, thus leading to the transcriptional activation of NF-AT, AP-1 and NF-κB transcription factors at a similar extent [6,8,112,121]. As a consequence, for several years, *in vivo* mouse models have been used for understanding the mechanisms of T lymphocyte activation and differentiation and the role of CD28 costimulation in health and immune diseases. Thus, when CD28 superagonistic antibodies (CD28SAbs) were discovered to preferentially activate and expand immunosuppressive regulatory T cells [122], an enormous amount of pre-clinical experiments have been performed to evaluate the potential use of these CD28SAbs to ameliorate the onset, progression and clinical course of human autoimmune diseases (Table 1). However, when a humanized CD28SAb (TGN1412) was administered to volunteers on March 2006, the phase I clinical trial turned in a catastrophe, because this antibody induced a rapid and massive cytokine production (i.e. IFN-γ, IL-1, IL-6, TNF-α), thus causing a severe systemic inflammatory response syndrome [123]. Altogether, the above reported data evidenced that the translation of experimental results from mice to men could determine dramatic effects, supporting differences in CD28 signalling capabilities between human and mouse [124,125].

The comparison of the sequence in the cytoplasmic tail of CD28 between human and both mouse and rat reveals a single amino acid substitution within the C-terminal proline rich motif: $\mathbf{P}^{212}$ in human CD28 Vs $\mathbf{A}^{191}$ in mouse and rat (Fig. 3). Although, this mutation does not affect the proline consensus sequence for SH3 binding, it may change a putative SH2 binding consensus sequence. For instance, $\mathbf{Y}^{209}$AP$\mathbf{P}^{212}$ within the C-terminal proline rich motif of CD28 is quite similar to different consensus sequences identified as optimal binding sites for the SH2 of Vav1 [126–130]. Our data on the loss of CD28/Vav1 interaction by mutating the tyrosine residues within the C-terminal proline rich motif of CD28 [43] together with our recent observations that mutation in the SH2 domain of Vav1 or P^{212} to A substitution within CD28 impairs both NF-κB activation and pro-inflammatory cytokine expression (Tuosto's personal communication), strongly support a role for YAPP sequence and Vav1 binding in mediating the functional differences observed between human and mouse CD28 (Fig. 3).

In conclusion, despite the enormous progresses made on CD28 costimulatory functions in T lymphocytes, the molecular mechanisms of CD28 signalling are not completely understood. A deeper understanding of the signalling properties of human CD28 will provide new avenues for the design of appropriate therapeutic drugs targeting CD28-associated signalling pathways for autoimmune/inflammatory disorders.

Conflict of interest

The authors have no financial conflicts of interest.

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