

REVIEW

Distinct NF- κ B activation pathways engaged by T-cell receptor and co-receptor CD28 on T-cells

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The transcription factor nuclear factor- κ B (NF- κ B) is critical for the induction of inflammatory responses in T-cells, survival and differentiation. Antigen receptor (TCR) and co-receptor CD28 are the central regulators of NF- κ B activation in T-cells. Progress in understanding NF- κ B activation in T-cells has occurred over the years with the identification of individual adapters such as ADAP and GRB-2 and enzymes such as PKC- θ that regulate NF- κ B. However, little is known whether the engagement of distinct modules by the TCR and CD28 account for the cooperative effects of the two receptors in activating NF- κ B. In this review, we discuss recent advances in our understanding of NF- κ B regulation by TCR and CD28.

Keywords: Adaptors; ADAP; VAV-1;GRB-2; NF- κ B

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Introduction

Nuclear factor- κ B (NF- κ B) includes a family of transcription factors that act as dimers and regulate genes involved in the inflammatory and immune responses, growth, development and differentiation. NF- κ B family is comprised of five members: RelA (p65), RelB and c-Rel, and the precursor proteins NF- κ B1 (p105) and NF- κ B2 (p100), which are processed into p50 and p52, respectively ^[1]. Activation signals from antigen receptors, inflammatory cytokines and infections all lead to the processing of p105 and p100 and their dimerization with RelA component. Complex of NF- κ B dimers bind to the κ B sites on the promoters or enhances of various target genes leading to the activation or repression of a specific signaling pathway. Examples include the IL-2 promoter that concurrently binds to transcription factors NF- κ B, NFAT and AP1 leading to the regulation of its expression during an inflammatory response ^[2]. Defects in the of NF- κ B pathway is linked to the immune

disregulation such as inflammatory disorders, autoimmune diseases as well as cancer ^[3,4]. The NF- κ B pathway therefore is tightly regulated at multiple checkpoints, and various receptors including CD28 are believed to use individual signalling components for its regulation. In resting T cells NF- κ B dimers remain bound to inhibitors of κ B (I κ B) molecules. Stimulation by antigen receptors and co-receptors induces IKK (I κ B Kinase) complex activation which is responsible for I κ B phosphorylation leading its degradation via ubiquitin pathway. IKK consists of three subunits, two catalytically active kinases IKK α , IKK β and a regulatory/structural subunit, NEMO ^[5]. The main consequence of I κ B degradation is the liberation of NF- κ B dimers from cytoplasmic retention pool and subsequent translocation to the nucleus. However, a second phase of NF- κ B regulation occurs in the nucleus which includes post-translational modifications of NF- κ B subunits to fine tune their activity on promoters and enhancers of multiple genes ^[1,6]. These regulatory steps are common to all cell

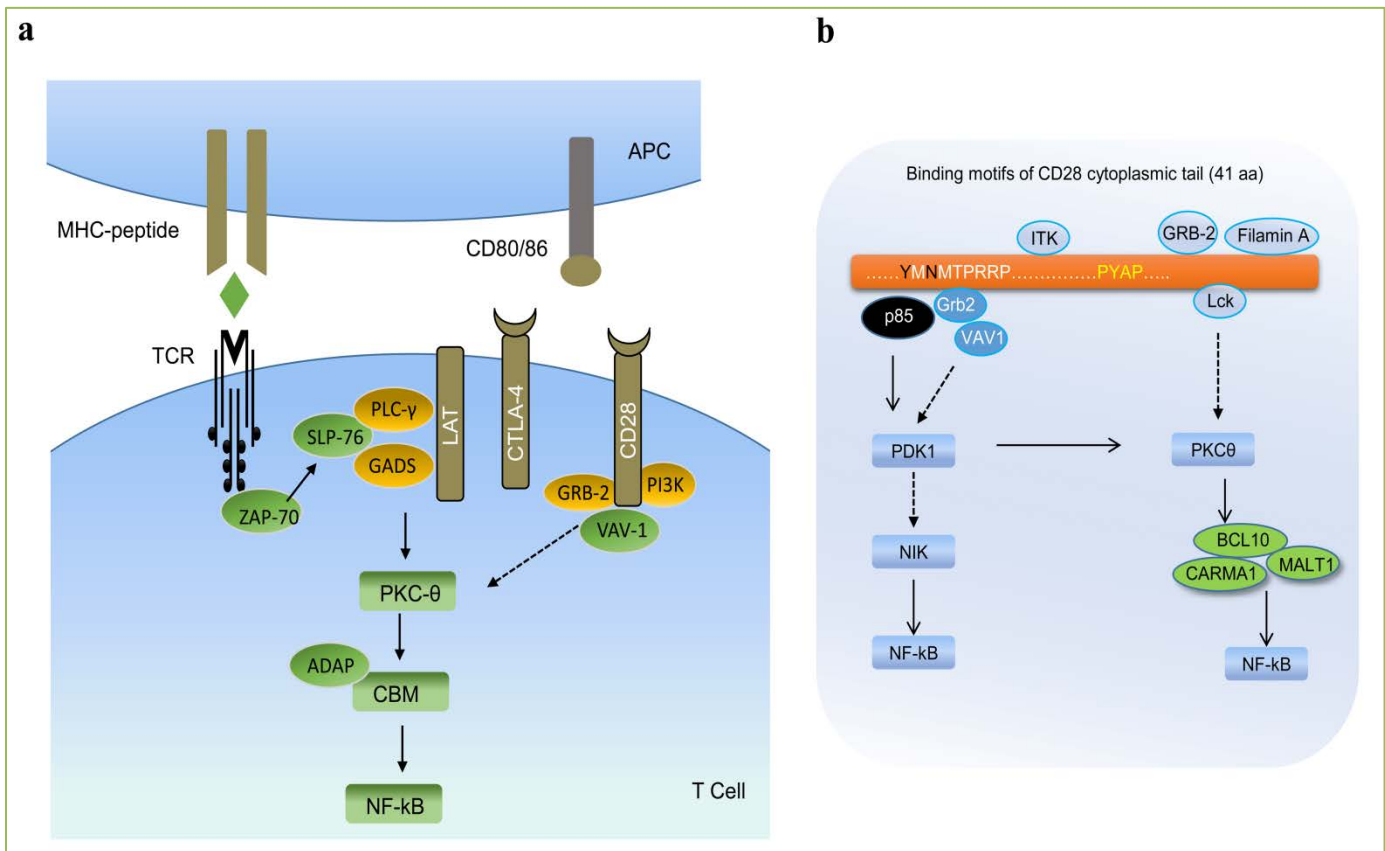


Figure 1. NF-κB activation in T-cells: a) upon engagement of the antigen receptor (TCR) by peptide-MHC, proximal signaling events activate tyrosine kinase, zeta-chain-associated protein kinase 70 (ZAP-70). ZAP-70 phosphorylates SLP-76 and LAT thereby recruiting several proteins resulting in the formation of a signalosome complex responsible for the activation of several downstream targets including protein kinase C theta (PKC-θ). PKC-θ is essential intermediate that couples TCR signals to NF-κB via its association with I kappa B kinase (IKK) and activation of Carma1-Bcl10-Malt1 (CBM) complex. IKK complex phosphorylates I kappa Bs (IκBs), leading to their polyubiquitination, release of NF-κB dimers and their translocation into the nucleus. ADAP is a scaffold protein that regulates CBM complex assembly upon TCR ligation and is required for TCR mediated NF-κB signaling but not CD28 pathway [23,25]. CD28 co-stimulation dependent NF-κB activation, on the other hand, is dependent on the binding of GRB-2 to its cytoplasmic tail. **b)** GRB-2 binds to the YMNMF motif in the cytoplasmic tail of CD28, and a point mutation (N-Q) that selectively disrupts this binding, abrogates CD28 driven NF-κB activation. VAV1 is required for CD28 NF-κB activation likely via its association with IKKα and GRB-2[23,39]. CD28 engagement also facilitates activation of PDK1 and its binding to the PKC-θ to activate NF-κB via classical pathway. CD28 ligation has been shown to activate alternate-like NF-κB pathway by recruiting p52/RelA dimers in a Vav1 dependent pathway, however, precise mechanism remains unclear [45]. In this situation, NIK, which is required for the alternate pathway is likely to be activated via PI3K-PDK1-AKT1 pathway upon CD28 engagement. PKC-θ also associates with CD28 in the immunological synapse via Lck, and regulates its localization and downstream signaling [46]

types including immune cells. However, NF-κB activation in T-cells is orchestrated by various unique signalling receptors and their potential crosstalk in the regulation of NF-κB has been unexplored. Proximal events upstream of IKK complex especially role of adapters in T-cells and NF-κB activation will be main subject of this review briefly touching on the other aspects of NF-κB regulation in T-cells.

Adapter molecules in T-cell activation

TCR and CD28 generate digital biochemical signals which are amplified and converted into effector functions in a series of well-defined signaling pathways [7-10]. One of the earliest events of antigen ligation is the activation of src and syk family kinases such as Lck and ZAP-70 respectively,

phosphorylating various enzymes and adapter proteins. Adapters are a unique group of proteins that have well defined structural domains but lack enzymatic activity [9,11]. Instead, they function as scaffolds bringing other proteins into the proximity of each other, nucleating and bridging multi-molecular signaling complexes [8,12]. Adapters can also induce conformational changes in their binding partners, potentially capable of regulating their activity and function [11,12]. Adapter scaffolds can regulate T-cell function in a positive or negative manner dependent upon the signaling complex and pathway. The importance of adapters in immune cell function and development has been underscored by *in vivo* and cell-line approaches, leading to important insights of their role in the transmission and integration of early signaling events generated by antigen receptor (TCR)

to downstream effector functions. Several adapter for these functions are exclusively expressed in the hematopoietic cells, while others are more widely expressed^[12]. Characterization of key positive adapters in T-cells has helped in our understanding of T-cell activation mechanisms and regulation. Key positive adapters include: SH2-DOMAIN CONTAINING LEUKOCYTE-SPECIFIC PHOSPHOPROTEIN (SLP-76), mice null for SLP-76 have severe disruption in thymic T-cell development and IL-2 production^[13-15]; LINKER FOR T-CELL ACTIVATION (LAT) is a transmembrane adapter with multiple cytoplasmic tyrosine phosphorylation/binding sites, an important signaling node to nucleate multi-protein complex of GADS, SLP-76, PLC-gamma, ITK and GRB-2. LAT or SLP-76 null mice displayed similar phenotype and a complete block in thymopoiesis at the pro-T3 stage^[16]. In addition to this, Jurkat T-cell mutant lines lacking either SLP-76 or LAT expression showed a vital requirement of both molecules in mediating TCR-induced PLC γ 1 phosphorylation, extracellular signal-regulated kinase (ERK) activation, Ca²⁺ influx and IL-2 promoter activity^[13,17,18]; GRB-2 RELATED ADAPTER DOWNSTREAM OF SHC (GADS) is a SH2- and SH3-domain-containing adaptor protein and plays crucial role in TCR-mediated signalling by linking LAT with SLP-76 adapter, thereby coupling membrane-proximal events to downstream signaling pathways. GADS-null mice revealed impaired T-cell development, with specific defects in both positive and negative selection of thymocytes^[19]. GADS also associates with the serine/threonine kinase hematopoietic progenitor kinase-1 (HPK1) which has been implicated in the activation of the JNK pathway^[20]. Adapters such as ADAP and SKAP1 with critical roles in the regulation of T-cell adhesion, viral transmission, NF- κ B regulation is described in detail elsewhere^[10,23,24,25].

Negative regulators of T-cell activation include PHOSPHOPROTEIN ASSOCIATED WITH GEMS (PAG), a transmembrane adapter protein that binds Csk (c-terminal Src kinase) which phosphorylates and inactivates src kinases (e.g. Lck) in resting cells^[21]. Other adapters that negatively regulate T-cell function is SH2-INTERACTING TRANSMEMBRANE ADAPTOR PROTEIN (SIT) by binding to Csk and protein tyrosine phosphatase 2 (SHP-2) enzymes^[22].

Role of adapters in NF- κ B activation

Generation of knock-out mice deficient for individual signalling proteins, together with biochemical functional studies, have identified several adapter proteins required for the co-stimulation induced NF- κ B activation^[26]. These initial reports also showed that co-stimulation induced NF- κ B activation was dependent on the initial tyrosine

phosphorylation cascade that engages adapters in multiprotein complexes containing several proteins required for NF- κ B activation such as Src homology 2 domain-containing leukocyte phosphoprotein 76 (SLP-76), growth factor receptor bound protein-2 (GRB-2), GRB-2 related adapter downstream of Shc (GADS), adhesion and degranulation promoting adapter protein (ADAP) and proteins with enzymatic activity as well as adapter-like functions, such as phospholipase C (PLC) γ and the exchange factor Vav1^[27]. While Vav1 contributes to Rac-dependent reorganization of the actin cytoskeleton, activated PLC γ 1 activates protein kinase C (PKC) to generate diacylglycerol (DAG) and IP3. Adapter SLP-76 has no intrinsic enzymatic activity but its expression is required for the activation of NF- κ B as shown in Jurkat cells J14 deficient in SLP-76^[28]. A role for SLP-76 *in vivo* has been less certain given that there are fewer mature T-cells in the periphery due to a severe block in thymic development at double negative (DN) stage^[14,15]. Disruption in the thymic T-cell development as a consequence of defective NF- κ B could not be ruled out. GRB-2 on the other hand has been demonstrated to directly associate with CD28 cytoplasmic tail^[29,30], and its role in the activation of NF- κ B pathway has been examined previously in Jurkat cells^[31], showing its expression is required for the CD28 linked NF- κ B pathway. In our recent study, we demonstrated that GRB-2 is an essential component of CD28 pathway and is vital for achieving full NF- κ B activation while intriguingly, it did not participate directly in the TCR driven pathway^[23]. Another study claimed a role for GADS in this pathway^[32], however, we have consistently observed stronger association of GRB-2 but not GADS with CD28^[23]. The point of CD28/TCR convergence of NF- κ B activation remains unknown.

CD28 and NF- κ B activation

Signals from co-receptors, particularly CD28 appear to cooperate with primary TCR signaling for optimal T-cell activation^[33]. NF- κ B pathway is no exception, and CD28 is a crucial component to achieve full immune response as demonstrated by CD28 KO cells that have diminished IL-2 production and activation. However, despite the importance of CD28 in potentiating TCR activation of T-cells (i.e. co-stimulation), increasing evidence has shown that its ligation alone can induce signaling events in T-cells^[34,35]. This is further supported by studies on the use of mitogenic CD28 antibody which can induce proliferation and cytokine burst in the absence of TCR ligation^[36]. We previously showed that GEF VAV1 binding to CD28 involves the intermediate binding of another adaptor GRB-2^[29,30]. Other investigators have shown that the loss of either GRB-2 or GADS binding to CD28 can abrogate NF- κ B activation in Jurkat T-cells^[37]. In addition, Tuosto *et al.* have reported

that non-mitogenic anti-CD28 can deliver a unique signal leading to the recruitment of p52/Rel-A complexes on Bcl-xL promoter^[38]. They showed that CD28 can co-operate with VAV-1 to activate NF- κ B in a pathway involving Rac-1 and mitogen-activated kinase kinase 1^[35,39].

On the other hand, Ca²⁺-independent PKC subfamily member PKC- θ predominantly expressed in T-cells has important and non-redundant roles in T-cell activation particularly via regulation of NF- κ B^[40,41]. The activity of PKC- θ is essentially regulated by its membrane localization and conformational changes^[42]. Recently, a conserved proline-rich motif in the V3 domain of PKC- θ was found to be required for association with CD28 and its immunological synapse localization and downstream effector functions^[43]. Thus, CD28 directly engages proximal pathways leading to the NF- κ B activation in T-cells.

New insights into the regulation of NF- κ B by CD28 and TCR

Despite the progress made in understanding NF- κ B regulation in T-cells, studies dissecting the individual components of TCR and CD28-mediated NF- κ B activation in primary T-cells have been lacking. In our recent paper, we addressed this issue by using primary T-cells from various knock-out (*Cd28*^{-/-}, *adap*^{-/-}) and knock-in (i.e. *Cd28* Y-170F) mice in conjunction with transfected Jurkat T-cells and showed that the TCR and CD28 use distinct pathways for the activation of the NF- κ B pathway in T-cells^[23]. CD28 engaged GRB-2 via YMNM motif in its cytoplasmic tail, which was required for NF- κ B induction. Using Y170F knock-in mice, NF- κ B activation was significantly dampened, so was the case when endogenous GRB-2 was depleted. CD28 induced NF- κ B pathway was further delineated by showing Vav1 as an important component of CD28/GRB-2 pathway. Vav1 expression significantly up-regulated NF- κ B, and its depletion abrogated NF- κ B activation in response to CD28 engagement. Surprisingly, CD28 KO primary T-cells had normal NF- κ B response when engaged by anti-TCR antibodies. Further, CD28 activated NF- κ B pathway was fully functional in ADAP deficient primary cells but defective TCR pathway. In both cases (ADAP or CD28 deficient primary cells), synergy in NF- κ B co-ligation of CD3 and CD28 was lost. The independent nature of CD3 and CD28 pathways in NF- κ B activation was also supported by results from LAT deficient cells showing normal CD28 activation, but no activation via TCR/CD3 pathway. Our findings provide evidence that the CD28 and TCR pathways regulate NF- κ B activity via different signaling modules of GRB-2/VAV1 and LAT/ADAP respectively.

Concluding remarks

In lymphocytes, NF- κ B controls expression of diverse set of genes involved in the productive immune response, division and growth. NF- κ B has been topic of intense study for several decades, and recent years have witness, complex interplay of signaling pathways that shape the spatial and timely outcome of NF- κ B activation^[44]. To successfully interfere NF- κ B pathway in clinical settings, receptors as well as co-receptor (stimulatory/inhibitory) engaging NF- κ B pathway entail further understanding. In this direction, we have uncovered that CD28 and CD3 use unique signaling modules of GRB-2/Vav1 and LAT/ADAP to achieve full activation of NF- κ B in T-cells respectively.

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