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Regulation of NF-κB induction by TCR/CD28

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Abstract

NF-κB family transcription factors are a common downstream target for inducible transcription mediated by many different cell-surface receptors, especially those receptors involved in inflammation and adaptive immunity. It is now clear that different classes of receptors employ different proximal signaling strategies to activate the common NF-jB signaling components, such as the IKK complex. For antigen receptors expressed by T and B cells, this pathway requires a complex of proteins including the proteins Carma1, Bcl10, and Malt1. Here, we discuss some of what is known about regulation of these proteins downstream of TCR/CD3 and co-stimulatory CD28 signaling. We also discuss another unique aspect of TCR-mediated NF-κB activation, i.e., the spatial restriction imposed on signaling events by the formation of the immunological synapse between a T cell and antigen-presenting cell presenting specific peptide/MHC.

Keywords

T cells; NF-κB; Signal transduction

Getting from TCR/CD28 to the IKK complex

General signaling pathways activated by the T-cell receptor for antigen (TCR) have been reviewed extensively elsewhere [1, 2], but here, we provide a brief overview, in order to establish context. Proximal signaling downstream of the TCR is mediated by the associated CD3 (γ, δ, and ε) and ζ chains, which contain conserved motifs of the ITAM (immunoreceptor tyrosine-based activation motif) family. Tyrosines within these motifs are phosphorylated by the src family kinases lck and fyn, leading to the recruitment of the Zap70 tyrosine kinase. Zap70 goes on to phosphorylate a number of downstream substrates, including the trans-membrane adaptor protein LAT and phospholipase C-gamma1 (PLC-γ1), which catalyzes the production of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). These second messengers then induce the release of intracellular calcium and activation of protein kinase C (PKC) and Ras guanine nucleotide releasing protein (Ras-GRP), respectively.

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Linking the TCR to NF-κB

It is the DAG-dependent arm of the TCR signaling cascade that is thought to be the main pathway by which NF-κB becomes activated. Thus, one target of the second messenger DAG is PKC θ, which is the predominant PKC isoform activated downstream of the TCR [3, 4]. Approximately 10 years ago, it was demonstrated that PKC h is critical for induction of NF-κB-dependent transcription after TCR engagement [5]. This followed closely on the heels of the identification of PKC h as a principal component of the immunological synapse [6], which is discussed below. The direct target for PKC in the activation of NF-κB was not known, until after the identification of an important set of intermediate proteins—Carma1, Bcl10, and Malt1—sometimes referred to as the "CBM" complex. Thus, two groups published simultaneously that the membrane-associated guanylate kinase (MAGUK) and caspase recruitment domain (CARD) protein Carma1 (sometimes referred to as Card11) are required for the activation of NF-κB by the TCR (and BCR), but not by the TNF-α receptor [7, 8]. Further investigation revealed that antigen receptor engagement resulted in the recruitment of the Bcl10 and Malt1 proteins to Carma1 in activated cells [9]. Various lines of investigation converged when it was discovered that PKC θ can directly phosphorylate Carma1 in a region known as the "linker domain" [10, 11]. Thus, it was demonstrated that PKC h phosphorylates several sites within the domain downstream of the N-terminal CARD, but upstream of the MAGUK domain, apparently allowing the adoption of an open conformation of Carma1. The current model is that the CARD of this phosphorylated, putatively open, form of Carma1 can interact with the CARD of the partner protein Bcl10, resulting in recruitment of Bcl10 and its associated Malt1 to the plasma membrane [12–14].

Although PKC θ appears to be the most critical kinase for phosphorylation and "activation" of Carma1 after TCR stimulation, other kinases have been shown to participate in this process. For example, CD28, through PI3K-generated PIP3, recruits PDK1, which then is able to efficiently bind to both PKCθ and Carma1 [15]. Carma1 is also required for Akt-mediated NF-κB activation in T cells [16]. Future studies are required to establish whether these kinases modulate NF-κB activity by phosphorylating Carma1. Moreover, the downstream kinase IKKβ contributes to formation of the CBM complex by mediating phosphorylation of Carma1. Thus, activated IKκb modifies the upstream signaling complex through a feedback mechanism, thereby optimizing the strength and duration of NF-κB signaling [17]. However, phosphorylation events may also suppress Carma1 activity. In this regard, it has been demonstrated that CK1α specifically phosphorylates Carma1 at S608, which impairs its ability to activate NF-κB [18].

Role of the CBM complex

Once the CBM complex is formed, how does it promote activation of the IκK complex, which then carries out the direct phosphorylation of IκB? One of the key events in the activation of the IKK complex is thought to be K63-mediated ubiquitination of the adaptor protein IKKγ/NEMO, which is found as part of a tripartite IKK complex that also contains the catalytic IKKα/β proteins [19]. Activation of IKK is also dependent upon phosphorylation of the catalytic subunits, which is carried out by the TAK1 kinase, normally found in a complex with the TAB 1 adaptor protein [20]. What is clear at this point is that the CBM complex is required for the inducible K63 ubiquitination of IKKγ/NEMO, but is

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dispensable for the inducible phosphorylation of the IKK catalytic subunits [21]. This suggests that the activity of TAK1 is controlled by a different mechanism. In any case, the Carma1 and Bcl10 proteins both appear to act as adaptors, since they have no defined catalytic activity. Certainly, the job of Carma1 fits that description since, in its open conformation, it recruits the Bcl10 and Malt1 proteins to the plasma membrane. As discussed above, Bcl10 contains a CARD domain, but no other obvious functional domains. Malt1, on the other hand, does possess catalytic activity; it is usually referred to as a "paracaspase" because of its homology to the classical caspase proteins [22], although it took some time to prove that Malt1 indeed contains protease activity [23]. Furthermore, this activity is important for NF-κB activation by the TCR, since a peptide inhibitor of Malt1 impairs antigen receptor-dependent activation of NF-κB [23]. Somewhat paradoxically, however, Malt1 inhibition or knockdown does not affect activation of the IKK complex or downstream phosphorylation or degradation of IκB. Thus, one possibility is that Malt1 activity actually results in the removal of a downstream negative regulator of the NF-κB pathway.

In addition to the now canonical CBM components, a number of other proteins have been implicated in the control of NF-κB activation by the TCR. Given the importance of ubiquitination for activation of the IKK complex, it is not surprising that at least one E3 ubiquitin ligase has been found in complex with the CBM proteins. Thus, an elegant biochemical study by Chen and colleagues [24] revealed that the E3 Traf6, which had previously been implicated in innate immunity, co-purified with a higher order complex including the CBM proteins, in TCR-activated T cells. Traf6 associates with Malt1 in response to T-cell activation and can function as an E3 ligase for Malt1 in vitro and in vivo, mediating lysine 63-linked ubiquitination of Malt1 [25]. The involvement of Malt1-dependent caspase 8 (Casp8) activation in TCR-induced NF-jB activity is consistent with the finding that Casp8 is involved in positive regulation of NF-κB activity [26, 27]. Moreover, Casp8 and c-Flip are recruited into lipid rafts to associate with NF-κB adaptors after TCR activation [28].

PDK1 was proposed to play a dual role by regulating PKCθ-mediated recruitment of the IKK complex and the recruitment of CARMA1 to the PKCθ signalosome [29]. ADAP (adhesion- and degranulation-promoting adaptor protein) also appears to play a dual role in regulating recruitment of NF-κ=B signaling proteins to the PKCh signalosome, as distinct sites in ADAP are critical for the recruitment of TAK1 and CARMA1 to PKCθ [30, 31]. All these studies suggest that an as-yet uncharacterized signaling cascade may connect the CBM complex to IKK activation.

Role of co-stimulation in NF-κB activation by the TCR

The scientific literature reflects a long debate on the question whether the TCR and the co-receptors induce separate signal pathways (qualitative model) or whether the signaling routes employed by both receptor systems are entirely overlapping (quantitative model) [32]. Strong support for a quantitative view of co-stimulatory signaling comes from a microarray study showing that the TCR-induced expression of thousands of genes in primary T cells is amplified (or suppressed) to varying degrees by CD28 co-stimulation, but

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no new gene is induced by CD28 co-stimulation [33]. It was therefore proposed that CD28 functions as a quantitative modifier that is required to overcome signaling thresholds that are not attainable by ligation of TCR alone. Recruitment of PI3K is a key event for coupling CD28 to the IKK/NF-κB signaling pathway. PI3K-generated lipids serve as second messengers that bind various proteins harboring PH domains, including PDK1 and Akt. A large number of studies suggest that Akt is not an obligate component of CD3/CD28-induced NF-κB signaling, at least in T cells. However, Akt might function as a kind of "rheostat," which may modulate the strength and/or duration of NF-κB activation in these cells [16]. Several groups have demonstrated that quantitative effects on NF-κB activity can selectively impact the expression of selected subsets of NF-κB target genes [34–38]. NF-κB has great potential as a drug target for chronic inflammatory diseases or within chemotherapy regimens, so it will likely be beneficial to better understand the mechanisms underlying differential regulation of NF-κB target genes. Thus, we propose that at least a part of this mechanism lies with the modulatory effects of upstream kinases, such as Akt, that are not necessarily canonical members of the pathway.

Spatial regulation of NF-κB induction during T-cell activation

SMACs and microclusters

T-cell activation has always been an intriguing process to study from a cell biological perspective, due to the complex and restricted fashion in which T cells encounter their specific antigen stimulus, i.e., in the context of an MHC molecule on the plasma membrane of an antigen-presenting cell (APC). Understanding of T-cell activation by APC's as merely the result of a highly specific interaction between TCR receptor and its cognate MHC–antigen complex was advanced when new evidence was provided to suggest a more sophisticated mechanism. The fact that affinity of the TCR for its cognate peptide/MHC is quite low suggested that during T cell–APC interaction, other receptor–ligand interactions may be required to increase both T-cell sensitivity and T-cell activation. For example, T-cell activation by APC's is exceedingly inefficient in the absence of interactions between the integrin LFA-1 on the T cell and its ligand ICAM1 on the APC [39].

A great leap in our understanding of the cell biology of T-cell activation came in the mid-1990s, with the work of Kupfer and colleagues, who demonstrated the formation of a stereotypical structure at the interface of a T cell interacting with an APC presenting cognate peptide. This initial work conducted by Kupfer and colleagues with static imaging of fixed T cell–APC conjugates defined a structure that they termed the "SMAC." or supramolecular activation cluster [40]. This structure is organized to form a distinct central region (c-SMAC), containing high concentrations of the (TCR)-CD3, CD28 clusters, as well as the signaling intermediate PKC θ, which was discussed in detail above, and a peripheral adhesive region (termed the peripheral SMAC or p-SMAC) containing clusters of the LFA-1 integrin, which appeared to be acting to "insulate" the c-SMAC. A more detailed kinetic characterization was offered by the study of Grakoui et al. [41], which revealed that T cell–APC encounter is followed by the reorganization of receptors and intracellular proteins into clusters to form what this group termed the "immunological synapse." Thus, the TCR initially localized to small structures at the periphery of the synapse and then appeared to

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progressively relocate to its center, in an active process. This latter work involved live cell imaging of T cells contacting artificial lipid bilayer surfaces containing peptide/MHC and integrin ligand.

Despite the many studies that have helped to elucidate the requirements for SMAC formation, there are still a number of questions about its function. One proposed model suggests that formation of the c-SMAC is required for T-cell activation. The immune response to antigen is finely regulated by T cells, which are remarkably sensitive to antigen load. T cells are in fact able to respond efficiently to just a few peptide–MHC complexes presented by APCs [42]. However, these early studies on the SMAC/ immune synapse did not answer the question of where the very earliest TCR-dependent signaling events occur. Thus, numerous biochemical studies have shown that tyrosine phosphorylation can be detected within seconds of T-cell activation, yet the SMAC takes on the order of minutes to form [43]. It is now thought that the earliest signaling events after TCR engagement take place in small discrete structures that have been termed “microclusters,” which can eventually fuse to form a mature immune synapse, or SMAC [44–46]. TCR signaling microclusters can be observed by following the localization of the TCR/CD3 or associated ZAP-70, after engagement on either an anti-CD3 antibody-coated plate or planar lipid bilayer with peptide/MHC. Not surprisingly, these microclusters are also enriched in phospho-tyrosine and numerous signaling intermediates that function downstream of TCR/CD3 [44–46].

Spatial regulation of NF-κB activation in T cells

Despite the intensity with which both T-cell activation and NF-κB induction have been studied, there is still relatively little information regarding the spatial regulation of NF-κB signaling components during T-cell activation. One early study from Israel and colleagues reported that the IKK adaptor protein IKKγ, also known as NEMO, could be co-IPd with the CD3 complex after TCR engagement [47]. These investigators also provided some functional evidence that recruitment of NEMO to the TCR was functionally relevant, since a Zap70-NEMO fusion, which contained just the SH2 domains of Zap70, could reconstitute NF-κB activation in a Zap70-deficient Jurkat cell line, although other signaling pathways (e.g., signaling to NFAT) were not rescued. This and other papers also investigated the subcellular localization of NEMO in T cells by confocal microscopy, although none of these studies provided images of very high resolution [21, 47]. Nonetheless, the accumulated evidence suggested that NEMO was enriched at the SMAC, consistent with its reported association with the TCR/CD3 complex after activation. These findings were extended somewhat by examining the localization of NEMO in T cells lacking expression of Carma1. Thus, Lin and colleagues demonstrated that there was little to no effect of Carma1 deficiency on general SMAC recruitment of NEMO; however, there was significantly less NEMO co-localization with a lipid raft marker in the absence of Carma1 [21]. Similar findings were also reported by Hara et al. [48] using peripheral T cells from Carma1-deficient mice.

There have also been some limited reports that included confocal immunofluorescence imaging of upstream components of the IKK complex in T cells. Thus, like NEMO, Carma1

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itself can be recruited to the SMAC and to lipid rafts [49], and we also reported that this appears to be PI3k dependent, suggesting a role for Akt (or another PI3k-regulated kinase) in Carma1 phosphorylation and/or localization [16]. Similarly, Bcl10 has been reported to be recruited to lipid rafts after stimulation [48]. Bcl10 has also been observed to form poorly characterized large punctate or filamentous structures after T-cell activation, which one group has termed “POLKADOTS” [50]. Structure/function analysis suggested that formation of these structures correlates with Bcl10 activation of downstream NF-κB, although the cell biological nature of these structures is still obscure. Nonetheless, we have also observed these structures and confirmed that their appearance requires T-cell activation (LPK, unpublished data). Certainly, the best characterized upstream signaling component in this pathway is PKC h, which was introduced above. PKC h is a canonical marker of the c-SMAC and is critical for driving downstream activation of NF-κB, mainly through direct phosphorylation of Carma1.

Surprisingly, there is still no information in the literature regarding the relationship of the early forming TCR signaling microclusters to the upstream components of the NF-κB signaling pathway discussed here, including the CBM complex proteins and the IKK complex. Thus, it is still not known whether Carma1 or the IKK complex is even recruited to TCR signaling microclusters and, if so, what the respective kinetics are of the recruitment of these and other proteins in the NF-κB signaling cascade. One of the difficulties may be that these signaling microclusters are more difficult to image than the much larger SMAC/immune synapse, thereby limiting access to those investigators with the most sophisticated techniques and imaging equipment. However, it will likely be instructive to pursue a detailed kinetic study of the localization of CBM proteins and the IKK complex relative to the early forming TCR signaling microclusters, since such studies may illuminate previously unappreciated mechanisms of NF-κB regulation downstream of the TCR and CD28.

Biography

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