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Research Article

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The Role of Cd28 Costimulatory Receptors in Signalling Pathways

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Abstract

Naive T cells require extracellular stimulus signals through the T-cell receptor cascade (TCR). This TCR then recognized the antigens present on the molecules of the major histocompatibility complex (MHC) using the CD4 and CD8 receptor. Upon recognition, TCR- induced signal transduction propagates signals through different molecules and induced secondary signalling. Consequently, intracellular signaling cascades determine the characteristics of immune responses mediated by T- cells. Consequently, rigorous regulation of T-cell activation is crucial for T-cell homeostasis and appropriate immune responses. Dysregulation of TCR signalling can result in energy or autoimmunity. In this review article, we summarize current knowledge on the signalling pathways that govern how the TCR complex transmits signals into cells and the roles of effector molecules that are involved in these pathways.

Introduction

The bone marrow is the main source of origin of T lymphocytes and has reached maturity in the thymus. These naïve T cells are not capable of responding to infectious agents requiring activation to mediate immune protection. At the level of Immune Synapse (IS), the activation of naïve T cell requires the co-ordination of antigen-presenting cell (APC), a cell that presents an antigenic peptide of the infectious agent through a major histocompatibility complex (MHC) class I or II molecule (Figure 1). T-cell receptors (TCR) are the primary mediators that recognize the infectious agents presented by APC and activate T-cells by generating recombinant DNA sequences in the thymus. Each TCR is unique and responsible for the origin of specific T cell [1,2]. Recombinant functional T cell emerges from the thymus results into naïve T cell migrates through the secondary lymphoid system (Lymph and Spleen) and peripheral system. TCR is composed of α and β chains, which are specific to recognize the antigenic peptide of the infectious agent when it is, bound to the appropriate molecule of MHC I or II. Each TCR also associates with either a CD4 or CD8 co-receptor, and then these two molecules bind to MHC (class I for CD8 and class II for CD4), further stabilizing the interaction between the T cell and APC [3]. However, this interaction between TCR-MHC is low-affinity and requires a large number of TCR 'hits' so in the majority of cases the stimulation via TCR alone is unable to sustain optimal activation of naïve and memory T cells. This pathway called T-cell activation signal 1 or first and first signal of pro-activation [4,5] determines antigen specificity. However, T cells need a second signal that determines the activation threshold and the functional outcome of antigen-specific activation.

After receiving two signals, T cells become activated and play an essential role in the recognition and clearance of pathogens by producing cytotoxic T cells or providing signals to the B cells to produce antibodies, which plays important role in immune response [6,7]. T cells mature through a complex process of morphological, phenotypic and functional changes, and then migrate to the areas of draining lymph node T cells (Figure 1). However, they are specialized T cells of the human adaptive immune system, which function as key players in both maintaining immune tolerance against self-tissues and orchestrating a multicomponent immune attack on pathogenic foreign microorganisms. T- Lymphocytes can identify a specific foreign pathogen (non-individual antigens) during the antigen presentation process and generate specific responses to non-individual antigens or cells infected with a pathogen. Which other results in the elimination of pathogens from the human body by producing cytokines as well as cytotoxic T cells, which produces toxic granule like granzymes, perforins and induces the death of pathogen-infected cells. T-cells expand and retain their memory when they encounter a pathogen in the body, allowing for immediate recognition when the same pathogen

is reopened. Besides, an immune response against foreign pathogen depends on well-orchestrated co-operation between the innate and adaptive immune system, which also creates longterm specific memory and specializes to the same pathogen encounter to protect the host against future infection by mounting a rapid and robust response [8].

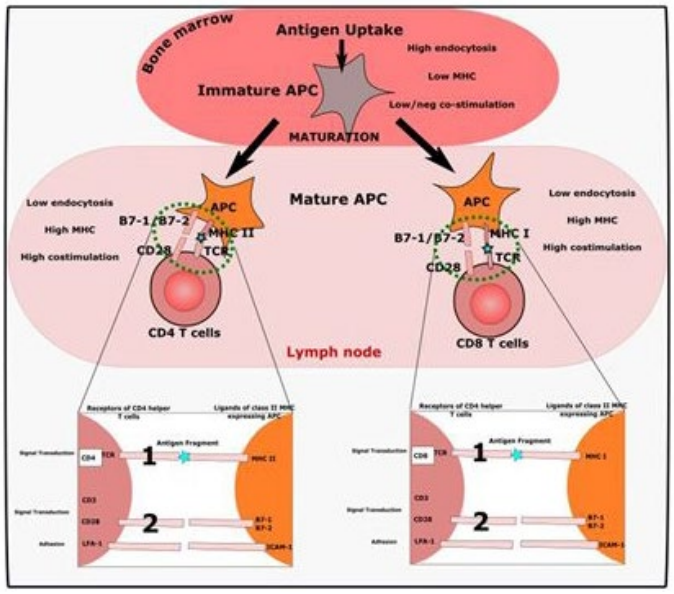

Figure 1: Maturation of APC in response to the lymph node pathogen: The immature cell interacts with an antigen and treats antigenic proteins. Cytokine receptor expression changes because of regulation of class II MHC molecule expression /concomitant stimulation and I. Migrates to the T-cell area of the lymph node draining tissue and presents T-cell peptides (CD4 T and CD8) with the appropriate CRT.

Primary and secondary signals are critical to the activation of T lymphocytes. However, the secondary signal is an independent co-stimulant biochemical signal for complete activation and survival [9,10]. The best- defined secondary signal is provided by the interaction of co-stimulatory receptor CD28 present in T cells and B7-1/CD80 or B7-2/CD86 present in APC [9,11]. The very first study of co-stimulation signal molecules in lymphocyte activation process explained by Cohn and Bretscher in a theory of self-non-self discrimination research article [12]. Following this, the importance of simultaneously transmitting a co-stimulator signal at the activation of the T-cell proposed by Lafferty and Cunningham [13]. Koller et al explained the co-stimulatory signal delivered in the absence of antigen recognition results in a neutral event for the T cell [14].

CD28 is a 44 kDa protein (Figure 2) is a glycosylated, disulfide-linked homodimer expressed on the surface of human T cells and it is also detected on plasma cells, neutrophils, and eosinophils, although the function is unclear [15-18]. CD28 express 80% on CD4+ and 50% on CD8+ (in Humans) and 100% both on CD4 and CD8 T cells (in mouse) [19].

Structure of CD28.

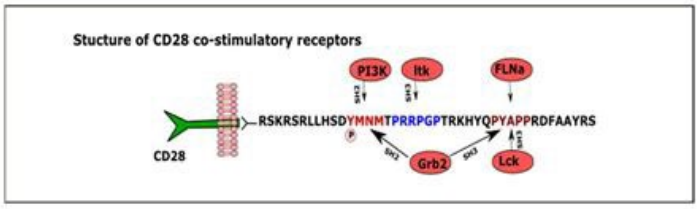

Figure 2: The co-stimulator signal CD28 initiates signal transduction by specific combinations of various proteins with cytoplasmic tail patterns CD28. Proteins that specifically bind to phosphotyrosine patterns or indicated non-hosphorylate patterns. Figure modified from Riha et al., 2010.

Engagement of CD28 on T lymphocytes improves metabolism, motility, actin cytoskeleton rearrangements, and cell cycle regulation, secretion of chemokines and cytokines, and activation of transcription factors. Moreover, CD28 costimulation has also involved in the T helper 2 (Th2) polarization, cytotoxic T cell differentiation, DNA repair, Signal transduction, gene expression, protein metabolism, apoptotic cells and prevent the anergy [20], and other functions of CD28 co-stimulation explained (Figure 3).

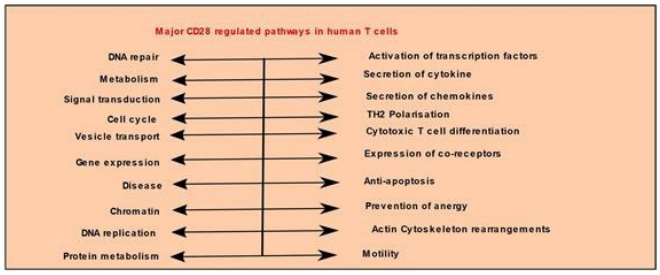

Figure 3: The secondary signal like CD28 plays a vital role in producing not only more functional and active cells but also other roles described in the figure. Figure modified from Riha et al. and Jonathan et al. [21].

The study of costimulatory receptors led to discover several other receptors and five classical family members which are structural homologs to CD28; Inhibitory costimulatory (ICOS), CTLA-4 (CD152), Program death-1 (PD1) and B and T lymphocyte attenuator (BTLA-17) [22,23].

These co-stimulatory receptors bind to respective ligands on APC surface:

- CD28-B7, CTLA-4-B7 [24].
- ICOS-B7h [25].
- PD-1-B7H-1/B7-DC [26].
- BTLA-17-HVEM (Figure 4) [23].

However, another group of co-stimulating molecules belongs to the superfamily TNF/TNFR but distinguishes itself from CD28 members with an elaborate cytoplasmic tail [27]. These receptors and bindings to respective ligands on APC surface: CD27: CD70,

GITR: GITR, 4-1BB:4-1BBL, OX-40: OX40L, and HVEM (B and T lymphocyte Attenuator, BTLA): Light/ LTalpha, CD30: CD30L [28,29]. Surface expression of CTLA-4, ICOS and BTLA are usually not reported on CD28null T cells in resting state, however, upon activation CD28null T cells are reported to express high levels of CTLA-4 on their surface [30,31]. PD- 1 and CTLA- 4 are generally considered inhibitory receptors linked to T cell exhaustion [32-34]. However, the expression of these receptors alone does not indicate that a T cell is exhausted, and despite the expression of these receptors in human T cells are reported to exhibit functional activity [35-37].

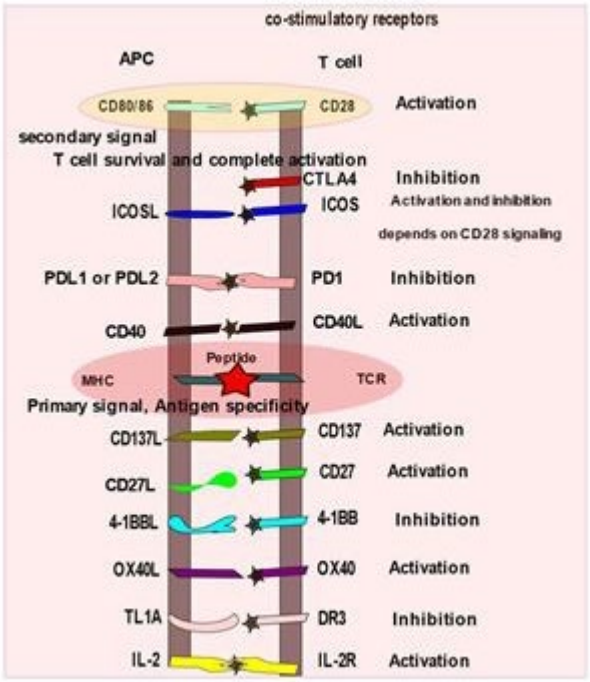

Figure 4: Paired interactions on the surface of an APC cell and a T cell that lead to T cell activation, cytokine synthesis and proliferation.

Molecular Signalling pathways

As a secondary signal molecule CD28 co-stimulatory receptor binds to CD80/86 ligand and activates PI3K, which further activates other signalling proteins that carry pleckstrin homology (PH) domains (contains 60+ proteins). This connection leads to the amplification of the TCR signals, by improving the adhesion due to the CD80/86 connection or in addition to the TCR signalling. Additionally, CD28 generates a determined signal against TCR signals that differ in intensity level. Therefore, secondary signal lowers the threshold for TCR activation of naïve T cells, allowing a response to low-affinity peptides and these responses did not observe in the absence of co-stimulation. However, high affinity peptides or chronic antigenic stimulation initiates activation without CD28 co-stimulation [21].

CD28-CD80/86 complex protein binding by using the MYPPPY motif leads to the initiation of costimulatory signal transduction cascade [38-40]. However, CD28, CTLA-4 and ICOS share a YMNM motif a consensus binding site for the p85 subunit of the lipid kinase PI3K [41-44]. CD28 composed of YMNM sequence, whereas having Asn residue in the +2 position and methionine at the +3 position confers additional specificity for Grb2/ GADS binding and p85 specificity respectively[45-47]. However, the ability to bind Grb2 is lacking in both ICOS and CTLA-4, which might account for the functional and signalling differences in these costimulatory receptors [41,40]. Previously, it has reported that the enigmatic tail of CD28 is tyrosine phosphorylated by the Src family kinases Fyn and Lck, as well as phosphorylated on serine/ threonine residues [48,49]. These specific protein-protein interactions induced by phosphorylation, PIP3 interactions with PH domains enable subsequent activation of downstream signalling cascades without novel protein synthesis.

PI3K is essential for surviving, activating, differentiating and migrating lymphocytes. According to their structural features and substrate specificity, PI3K was classified into four classes (IA, IB, II and III, Figure 5). Class IA PI3Ks were involved in activating surface lymphocyte receptors due to a heterodimer between a p85 regulating or adaptive subunit and a p110 catalytic subunit. The regulatory sub-unit p85 has been subdivided into five sub-units p85a, p55a, p50a, p85b or p55g and a catalytic sub-unit subdivided into p110a, p110b or p110g. However, p110a and p110b isoforms are expressed in all cells, while p110g is mostly expressed in immune cells. Class IA and IB activated by CD19, CD28, and ICOS costimulatory receptors; IL-2, IL-3, IL-7, IL-15, and granulocyte-macrophage colony stimulating factor (GM-CSF) cytokine receptors [50-54]. Class IB of PI3K deficiency affects mainly T-cell development and function. The CD28 PYMNM pattern binds to the SH2 domain of the regulatory subunit p85, facilitates the location of the catalytic subunit p110 in the plasma membrane, and leads to receptor activation. Phosphorylate kinases, phosphatidylinositol with phosphatidylinositol (3, 4)-biphosphate (D-3) lipids that combine with the internal leaf of the plasma membrane and allow the recruitment of proteins containing PH domains. Similarly, PI3K has a pletiotropic effect by activating multiple signaling channels. The basis of p85 recruitment by CD28 is with the same binding affinity as binding to growth factor receptors such as the platelet-derived growth factor receptor (PDGF-R) [44].

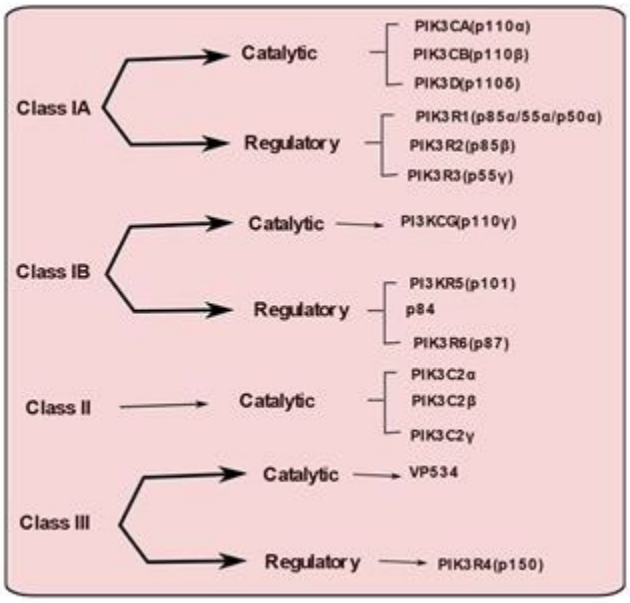

Figure 5: Classification of PI3K in relation to structural features and substrate specificity.

The high affinity and specificity link between the PIP3 and PH domains of the PI3K effector helps in recruiting and activating the plasma membrane. The PH domain is an evolutionary preserved structural fold found in proteins expressed in organisms ranging from yeast to mammals [55]. However, one well-established downstream pathway of PI3K involves the activation of phosphoinositide-dependent protein kinase 1 (PDK1), which in turn activates protein kinase B (PKB/AKT). PDK1 and PKB may themselves phosphorylater and regulate several pathways. PKB/AKT phosphorylation by PDK1 at threonine 308 the residue is involved in the regulation of protein synthesis, cellular metabolism and cell survival as activated PKB/AKT further phosphorylates BAD, caspase-9, transcription factors CREB1 (cAMP-responsive element-binding protein 1), Foxo, and glycogen synthase kinases-3a and b (GSK3a and GSK3b (Figure 6) [21].

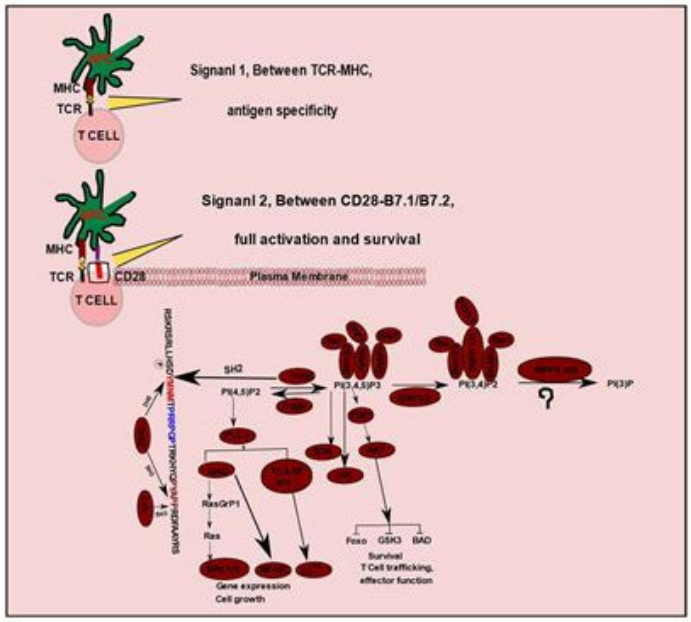

Figure 6: Regulation of different cellular responses by PI (3,4,5) P3 in plasma. Figure modified from Neetu et al. After the primary signal between TCR-MHC, PI3K is recruited via the SH2 domain at the level of the plasma membrane. PI3K converts PI(4,5)P2 to PI(3,4,5)P3, whilst PTEN and SHIP1/2 hydrolyse PI(3,4,5)P3 to PI(4,5)P2 and PI(3,4)P2 to PI(3,4,5)P3, respectively. PLACy converts PI (4,5) P2 into DAG and IP3. The DAG also activates the RasGrp1-Ras-ERK1/2 cascade, and IP3 implies a mobilization of ca 2. However, proteins from the PH domain PDK1, AKT, BKT, ITK regulate the secondary signal process leads to T activation, proliferation, survival and development. In addition, adaptive proteins from PH GABs, SKAPs, Bam32 and TAPP also contribute to the signaling process. AKT, protein kinase B, PDK1, phosphoinositide- dependant kinase-1, PLCy- phospholipase Cy, ITK, IL-2 inducible T cell kinase, and BTK, Bruton agammaglobulinemia tyrosine kinase.

PI(3,4,5)P3 recruits PH domains containing plasma membrane proteins and controls cellular responses. PI3K de-phosphorylates PI(4,5)P2 to PI(3,4,5)P3, which recruits PH domain- containing signal proteins to the plasma membrane, then PH domain-containing proteins are activated and involve in cellular responses such as cell growth, gene expression, effector function, and T cell survival.

Despite the regulation of various cellular responses, the role of CD28 related to PI3K remains uncertain. It might be related to TCR ligation itself or could increase PIP levels, which results in the unclear role of PI3K in T cell development and function [54-59]. In an experimental analysis, he showed that disruption of the gene (p85a encodes) affects T cells weakly, while strongly affecting B cell development and proliferation. Surprisingly, T cells lacking p85b gene has a high proliferation rate, despite completing the more cell divisions in both CD4+ and CD8+ T cells [60,61]

At the level of the immune synapse (IS), CD28 co-stimulatory signals regulated by guanine nucleated exchange factor Vav1, small Rho GTPase cell division control protein 42 (Cdc42), the Whiskott-Aldrich syndrome protein (WASP), and the actin-related protein 2/3 complex (ARP 2/3) [62-65]. Moreover, filamin A is significant to regulate the TCR and CD28 signalling. However, in the absence of filamina A, the CD28 co-stimulation was compromised and the membrane raft bundles were lowered at the SI. In addition, filamin A might be involved Vav1 dependent actin-polymerization pathway to regulate the raft clusters at the IS [66].

Vav1 regulates T lymphocyte responses and cytoskeletal dynamics. Vav1 deficient cells resulted in defects in cytoskeletal reorganization and T cell activation [67]. Vav1 controls T lymphocyte responses and cytoskeletal dynamics. Lacking the adaptor molecule cbl-b in T cells show increased Vav1 activation, CD28 independent triggering and membrane raft clustering at the IS [68, 69]. However, in the absence of cbl-b proteins results in dysregulated Vav1/WASP signaling and defects in membrane raft recognition in the presence of CD28 co-stimulation [68, 70].

PI(3,4,5)P3 plays a crucial role in developing and activating T cells. However, inositol poly- phosphatases (Phosphates and tensin (PTEN), SHIP1, SHIP2, and INPP4 A/B) may have downregulation or activation role in the downstream of PI3K in T lymphocytes (Figure 7) [71].

Impaired SHIP1 leads to severe myeloproliferative disorder, and the loss of NK cell function whilst PTEN deleted mice do not show T cell responses [72]. PTEN homology heterozygous mice show high incidence of tumours, develop autoimmune responses and Fas-mediated cell death. These mice T cells have increased the ability to proliferate, and reduced activation of cell death suggests that the crucial role of PTEN in T cell activation and survival [71]. However, inositol-phosphate 4- phosphatases enzymes available in two isoforms INPP4A and INPP4B has a specific role in removing phosphate at the four positions on the inositol ring to convert PI(3,4)P2 to PI(3)P. INPP4A is strongly expressed in the brain, while INPP4B is expressed in skeletal muscle, heart, brain, pancreas and epithelial cells of breast and prostate. INPP4A and INPP4B have shown that they regulate neuroexcitatory cell death and a powerful tumor suppressant in breast cancer, respectively. However, the role of INPP4A and INPP4B was not observed in immune cells, although INPP4B contributed to myeloid-derived osteoclast function and bone remodeling [71].

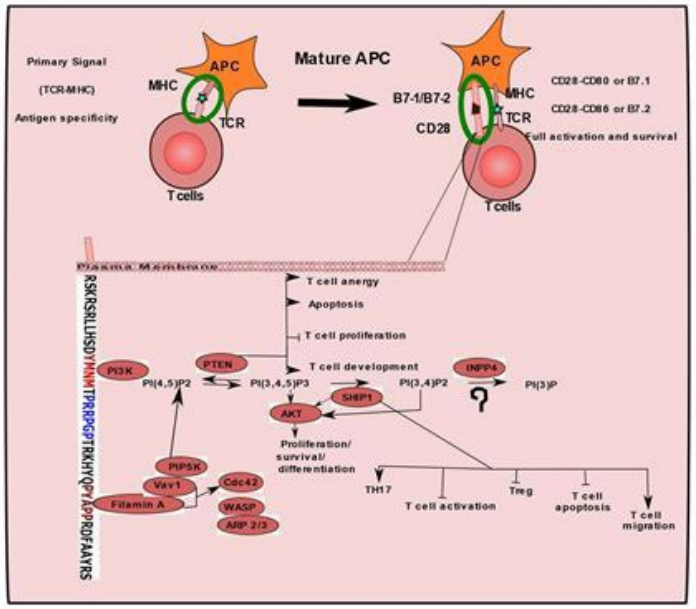

Figure 7: Role of innositolic phosphatases in T-cell activation. Figure modified from Neetu et al. During the primary signal between TCR-MHC, PI3K is activated to move to the membrane over the SH2 domain. At the PI membrane phosphate substrate (4.5), P2 converts to PI (3.4.5) P3. The SH2 domain associated with the AKT, PDK1, BTK, ITK, Vav and PLCy proteins, which further activate the secondary signal cascade, leads to T-cell activation, proliferation, survival and cytokine production. In the secondary cascade, PI(3,4,5)P3, PTEN, SHIP and PI(3,4)P2, recruited by AKT, plays a vital role in the development, proliferation and complete activation of T cells. However, the role of INPP4 remains to be confirmed.

Activation of the NFk B Transcription Factor

NFkB plays a role in regulating immune responses to infection and dysregulation of NFkB may be linked to inflammation, viral infection, autoimmune diseases and cancer. TCR signal alone is not sufficient, requires CD28 costimulation to activate the NFkB pathways [47,73]. However, PI3K plays a vital role in enabling PKB/AKT and other phosphorylates and activates IkB kinase (IKK) (Figure 8). IKK, in turn, inhibitor IkB phosphorylates, promoting its degradation, and thus facilitating NFkB translocation in the nucleus. Other potential paths also focus on PKC-θ as a central regulating node and include IL- 2-inducible T-cell kinase (ITK)-PLC-g1 activation, Grb2-Vav1, and FLNa. Downstream of PKC-θ is MAGUK (membrane-associated guanylate kinase) family member CARMA1 (CARD-MAGUK 1, caspase-recruitment domain-membrane-associated guanylate kinase 1) complex formation with BIMP1 (Bcl10-interacting MAGUK protein), Bcl10 and MALT1 (Mucosa-associated lymphoid tissue lymphoma translocation protein 1).

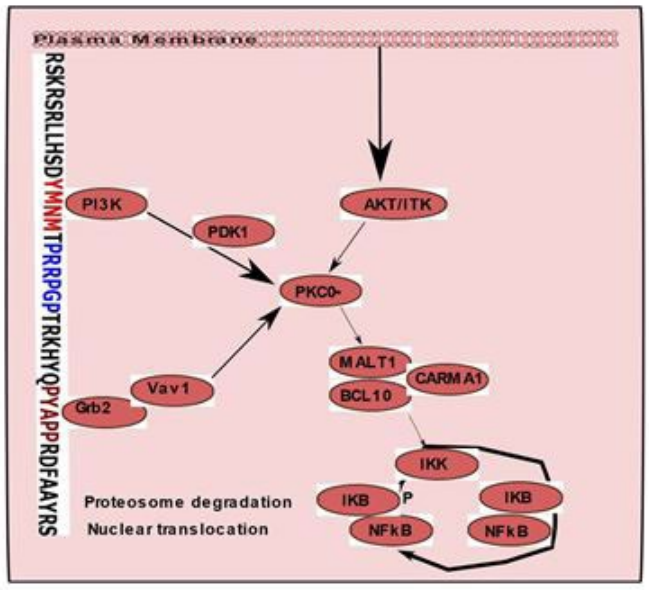

Figure 8: The CD28 NF-kB control pathways interacting with PI3K result in the production of D-3 lipids and the recruitment of PDK1 which phosphorylate and active PKB and PKC-θ. PKB may activate IKK by phosphorylating. Activated IKK Phosphorylates IkB, which disrupts its binding to NFkB target to degradation in the proteasome. Free NFkB can then carry out a translocation in the kernel to initiate the translocation. PKC-θ could also help with the assembly of the Bcl-10-CARMA1-MALT1 complex and subsequent activation of IKK. Figure modified from Riha et al.

Conclusion

The active TCR complex plays a vital role in T-cell-induced immune responses. Because of considerable advances in molecular, genetic and biochemical techniques, it is possible to elucidate the structure and signaling molecules of the TCR complex. The engagement of the TCR complex is a prerequisite for the launch of the TCR signal stunts summarized in this review. TCR signaling is essential for many aspects of the regulation of T lymphocytes, including development, differentiation, activation, proliferation and survival. Therefore, TCR signalling must be tightly regulated. In this regard, therapies have been developed that target the RCT complex, primarily for immune suppression.

Regulating TCR signaling is a complicated process controlled by a large number of effector molecules. There are still numerous aspects of T-cell activation and development that are not yet understood. The integration of CRT-induced signalling and CD28-induced signalling is fairly well understood. However, the effect of the imbalances between these two signalling cascades on the differentiation and function of T lymphocytes is not well understood. For example, strong differentiation of the CD28 Th17 signaling blocks. As a result, unknown regulatory mechanisms control T-mediated immune responses. A better understanding of these processes will enable us to modulate immune responses for the treatment of autoimmune diseases and other diseases associated with the immune system.

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