

Molecular insights into T cell development, activation and signal transduction (Review)

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Abstract. T cell modulation plays a fundamental role to adaptive and innate immunity, which aids the recognition and defense against pathogens while also maintaining self-tolerance. Numerous molecular pathways participate in this process including thymic selection, T cell receptor and antigen-presenting cells cross linkage, along with co-stimulatory signaling cascades. The present review demonstrates a holistic analysis of various classic and novel mechanisms that govern T cell regulation and emerging therapeutic applications. Recent advancements have introduced novel roles in the journey of T cell modulation that can have a pivotal impact on the understanding of this process; for example, phase separation of the linker for activation of T cells, and the newer application of chimeric antigen receptor (CAR) T cell therapy in autoimmune diseases. While discoveries of proximal and distal signal transduction pathways have contributed to the comprehension of T cell anergy, cytokine-mediated differentiation and the delicate balance between immune activation and tolerance, there are still unresolved debates about further molecular mechanisms. There are also still questions about the long-term side effects of CAR-T cell therapy. Deeper research and analysis are required to further aid the understanding and use of this novel therapeutic approach.

Contents

1. Introduction
2. T cell development
3. T cell activation
4. T cell signal transduction
5. Conclusion

1. Introduction

One of the major elements of the adaptive immune response is T cells, which lead to the recognition of antigens and response to foreign invaders, while simultaneously maintaining self-tolerance. T cell activation requires an intricate signaling pathway. It starts with the recognition of antigens by T cell receptors (TCRs) presented through a major histocompatibility complex (MHC) on the surface of antigen-presenting cells (APC), such as macrophages and dendritic cells. Self-tolerance is preserved by co-stimulatory signals, such as CD28. Finally, a third signal is initiated by cytokines which play a major role in T cell differentiation. Any disturbance in this pathway can result in serious sequelae, such as autoimmune diseases, immunodeficiencies and malignancies. Recent developments in immunology have focused on T cell pathways for therapeutic purposes, which has potential. However, mechanisms underlying T cell anergy and linker of activated T cells (LAT) phase separation, along with the modulation of T cells and its use in novel therapeutic approaches, remain incompletely understood. The aim of the present review was to provide an overview of T cell activation and regulation along with the main molecular pathways, with the introduction of novel mechanisms and newer medical interventions, which would help further fill the current gaps in research.

2. T cell development

Background. T cell development predominantly takes place during fetal development and early childhood, shaping the adaptive immune system for lifelong function (1). Therefore, the congenital absence of the thymus in DiGeorge syndrome,

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due to a microdeletion in chromosome 22, leads to improper development of the third pharyngeal pouch. Patients with this syndrome present with deficient cellular immunity or autoimmune diseases, such as idiopathic thrombocytopenia (2). During puberty, gland shrinkage takes place. T cell maturation and development diminish without affecting health, even when the gland is surgically removed (1).

Several immune-deficient mouse strains have been recognized. Nude and severe combined immunodeficiency (SCID) mice represent an example of complementary T cell-deficient strains. Nude mice (they are named so because they lack fur) are naturally mutated and are bereft of thymus glands (3). Consequently, T cell poverty ensues; nonetheless, they possess B cells. SCID mice have combined immune deficiency because they lack the recombination activating gene (RAG) enzyme that is important for somatic recombination (4). This occurs even though these mice have a well-developed thymus. The lymphoid progenitors normally thrive when transferred from nude to SCID mice, since they have an efficient thymic microenvironment. A more recent study has further advanced our understanding by showing that murine models play an essential role in dissecting interactions between thymic stromal cells and developing thymocytes. The aforementioned interactions are indispensable for the establishment of the immune scenery along with T cell selection. Therefore, using these models to further investigate thymic regeneration would be a viable option aiming to restore T cell function in human patients (5).

Initial stages of T cells development. Hematopoietic pluripotent stem cells, which reside in the bone marrow vascular niche, give rise to lymphoid progenitors and myeloid progenitors. The myeloid progenitors develop into the cells of an innate immune response. The lymphoid progenitors give rise to T and B lymphocyte precursors, which are adaptive immune components. B cell precursors stay in the bone marrow and pursue their development there. Concomitantly, the T cell precursors evolve in the thymus, reaching it through the blood from the bone marrow (6). Since T cell development takes place in the thymus, it is considered a primary lymphoid organ mimicking the bone marrow.

The process of T cell migration from bone marrow to the thymus is influenced by chemotactic agents, including thymosin, thymopoietin and thymic factors (7). T cell precursors enter the thymus through the high endothelial venule. Once they are inside, they migrate to reach the subcapsular area, where they proliferate and increase in number. As they descend from the cortical region to the medulla, they undergo several maturation steps to become mature naïve T lymphocytes. Developing T cells that fail the selection processes die by apoptosis (programmed cell death). The process of T cell development lasts ~3 weeks, after which they become mature naïve T-lymphocytes. The maturation stages of T cells can be recognized by the markers expressed on their surface.

Initially, T cells are devoid of CD4 and CD8 markers of their future lineage. Therefore, they are named double negative cells (DN). The DN stage is further divided into four substages: DN1, DN2, DN3 and DN4, demarcated by CD44 and CD25 expression. The former is an adhesion molecule, while the latter is an IL-2 receptor. At DN3, the pre-TCR

is formed by the pairing of a functional rearranged β chain with pre- α chain. The vast majority of TCRs in postnatal life are composed of α and β subunits, whereas γ/δ TCR is predominant in fetal T cell lineage (8). TCR receptor assembly commences with V(D)J recombination at the TCR β gene locus (9). Thereupon, the rearranged functional β chain binds to the pre- α chain, forming pre-TCR. Next, pre-TCR and CD3 engender vital signals for survival, proliferation and cessation of β chain rearrangement. In the bargain, these signals drive DN cells to become double positive (DP).

At the end of this process, mature TCR recognizes manifold antigens. This diversity of immune repertoire arises from variable diversity joining V(D)J recombination of the β subunit, and VJ recombination only of the α subunit (cut and paste rearrangement) for the TCR genes. V(D)J starts with DNA breakage engendered by RAG1/RAG2 at recombination signal sequences (10).

Positive selection processes. Double-positive T cells can interact with epithelial thymic cells that present MHCs: Class one and two (MHCI and MHCII). This process ensures MHC restriction. If the DP T cell does not bind to MHC within 4 days, the fox receptors on their surface will be activated to initiate apoptosis (11). The second positive selection takes place in the medulla of the thymus gland following the negative selection processes. Both T cell groups, that is, T helper (Th) or T-cytotoxic (Tc), are determined in the second positive selection process. Cells that interact with MHCI become Tc cells, while those that recognize MHCII develop into Th cells. Signals that are received through CD8 shut down CD4 expression; hence, the first received signal determines the fate of T cells. T cells that bind to MHCI and MHCII with equal affinity are eliminated. The absence of MHC expression impedes the development of the corresponding T cell lineage.

Recent studies highlight that bare lymphocyte syndrome, a severe immunodeficiency disorder, arises from defective MHC expression, leading to impaired antigen presentation and T cell maturation (12). Consequently, failure of T cell type production emerges. Mice that express defective CD8 that cannot bind to MHCI do not proceed to produce mature Tc cells. It has been reported that there is a gene that is involved in the development of multiple systems. This gene represents a mammalian equivalent of Notch, which directs the T cell to become a Tc cell (13). This gene is also of great importance for directing the lymphoid progenitor to form T cell precursors. Abnormality in this gene may cause a shortage of T cytotoxic cells (14).

Negative selection process. The lucky T cells that successfully pass the positive selection exam undergo a negative selection test before they finish their thymic training. This process ensures self-antigen tolerance (15). In negative selection, self-antigens are presented by APC to T cells. T cells that strongly bind to self-antigens undergo apoptosis to maintain self-tolerance; failure in this process contributes to autoimmunity (16).

This process is referred to as the central tolerance mechanism. The differential avidity hypothesis states that the MHC complex delivers both positive and negative selection signals; nevertheless, the signal avidity for negative selection is higher (more signal is required to save the cell from apoptosis (11).

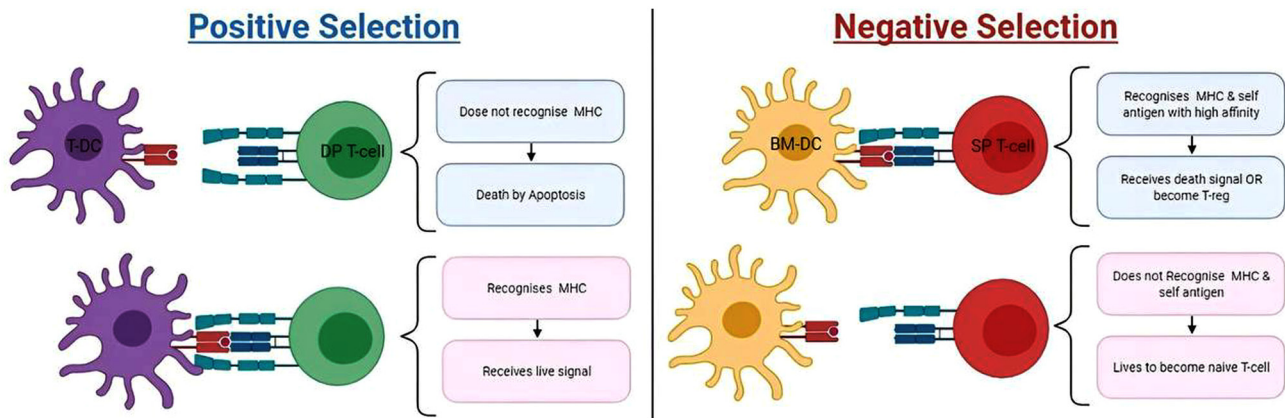


Figure 1. Positive and negative selection. MHC, major histocompatibility complex; DC, dendritic cell; BM, bone marrow.

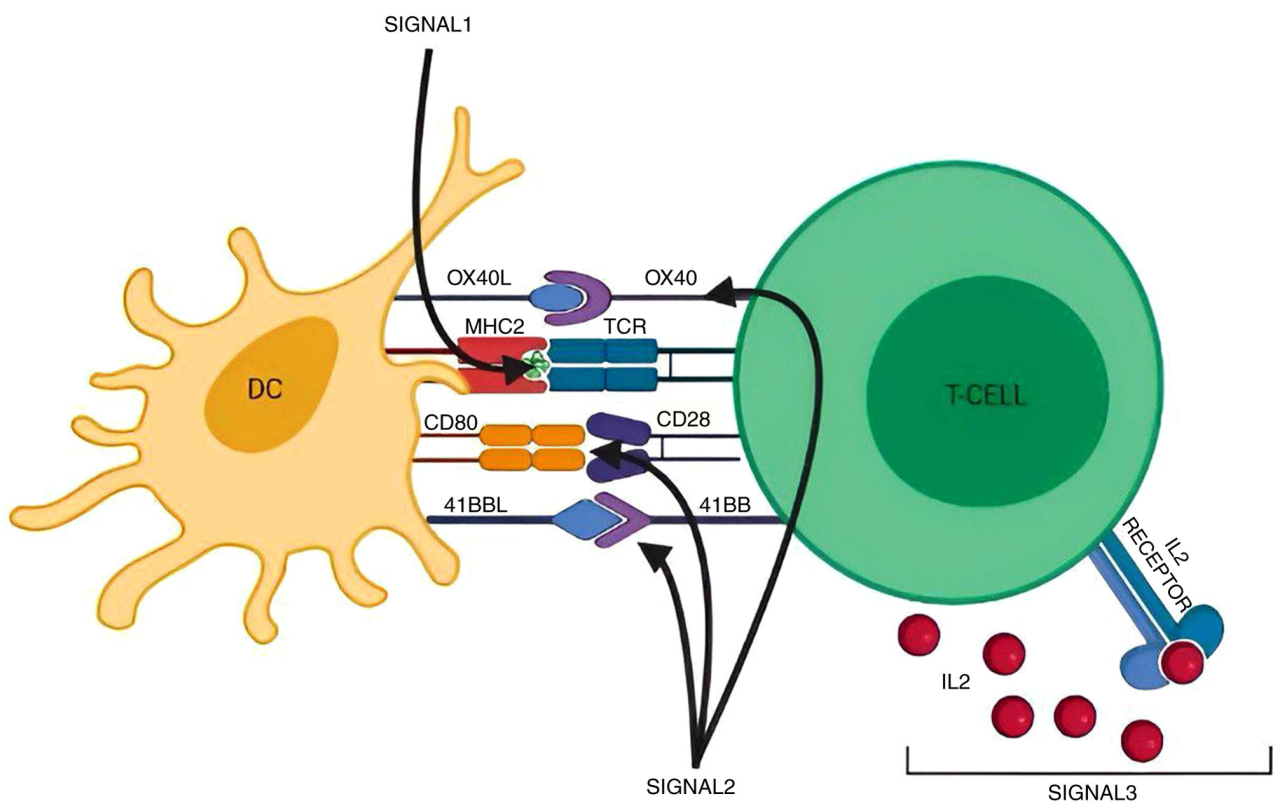


Figure 2. T cell activation signals. DC, dendritic cell; MHC, major histocompatibility complex; TCR, T cell receptor.

Next, mature naïve T cells will finally complete their stringent training and exit the thymus to pursue their function (Fig. 1).

3. T cell activation

Background. It is a process in which a naïve T cell gets activated to form a mature T cell. The T cell activation process undergoes rigorous control. Once activated, it proliferates and secretes cytokines that modulate immune response. Naïve Th cells engage with antigens bound to MHCII, while cytotoxic T (Tc) cells recognize MHCI-associated antigens, a critical step in adaptive immunity. Three signals are required for the activation of T cells. In signal one, the TCR binds to antigens presented by MHC on APC. Following the first signal, T cells

require co-stimulatory signaling to avoid anergy (a state of functional unresponsiveness to antigens, crucial for immune regulation). The final signal is the production of cytokines that stimulate T cell proliferation (Fig. 2) (17,18).

Signal one. This signal usually takes place in the secondary lymphoid organ for Th cells. The TCR binds to peptides presented to them by MHC molecules on the surface of APCs. The CD4 and CD8 molecules then interact with the beta chain of the MHC molecule, stabilizing the binding of TCR-MHC and helping in intracellular signaling (19).

Signal two. The co-stimulatory signal is mediated by an interaction between CD28 on the surface of Th cells and the

B7.1 (CD80) or B7.2 (CD86) proteins on the surface of APCs. This interaction actuates T cell proliferation. In addition, the interaction between CD28 and B7 effectuates the production of CTLA-4, which lessens the signal by competing with CD28 for B7 (20). This represents a negative feedback mechanism that wanes the immune response. Tc cells demand signals from co-stimulatory molecules other than CD28, such as CD70 and CD137. Other signals that are expressed on the surface of T cells only after the recognition of antigens include OX40, inducible T-cell co-stimulator and 4-1BB (21). These receptors bind to their ligands on APC, which are also not constitutively present on APCs.

This hinders the T cell activation bound to MHC in the absence of an antigen, which is the main role of these signals. The omission of these signals turns off T cell activation, and the dearth of signal two leads to anergy in naïve T cells (22), which means a reduction of immune response to a specific pathogen. T cells that develop anergy after TCR engagement are tolerant to that particular antigen (23).

Signal three. Immediately upon successful accomplishment of signal one and signal two, T cells encounter more signals to determine their responder phenotypes; these signals are cytokines. Th cells under the effect of IL-12 become Th1, under the effect of IL-4 become Th2, and under the effect of IL-23 and IL-16 become Th17 (24,25). Regarding Tc cells, signal three is mediated by IL-12 or adjuvant. Signals one and two alone can mediate Tc proliferation, in case of high antigen levels.

On the other hand, a low antigen level mandates the presence of signal three for cell proliferation to take place. Nevertheless, T cells that proliferate due to high antigen levels without the guidance of a third signal, fail to ensure cytolytic function (26). Hence, the fate of proliferated Tc cells depends on third signal exposure (25). Tc cells that lack their cytolytic function are considered tolerant (27). Out-of-order signaling may occur in certain settings. Usually, the third signal does not take place before the first two signals. This is largely attributed to low cytokine receptor expression in naïve T cells juxtaposed with their antigen-experienced fellow (28,29).

Signal three thwarting alters Th cell proliferation but does not affect that of Tc cells. When Th cells are exposed to high levels of cytokines, for example in therapeutic administration immunotherapy to treat cancer, ephemeral paralysis supervenes (30). This is largely due to the activation of the suppressor of cytokine signal 3 (COCS3) pathway. The COCS3 pathway hinders the piling up of Th cells quantity *in vivo* and *in vitro*, consequently affecting the adaptive immune response (31). Some studies showed that naïve Th cells may exhibit a response to cytokines despite the scarcity of Th proliferation (29,32). This response is mediated by activating factor upregulation or signaling molecule phosphorylation. The sequel of this response remains ambiguous and not fully understood. In recent studies, T cell modulation has been introduced in autoimmune disease management after it was widely accepted in oncological treatments. A trial was performed in the UK including patients with severe systemic lupus erythematosus (SLE) who received genetically engineered T cells to express chimeric antigen receptor (CAR) designed to target CD19 receptor present on autoreactive B cells. This technique is commonly known as CAR-T cell therapy. The trial showed

promising outcomes in halting the autoantibody release from autoreactive B cells. However, it poses some risks and drawbacks, such as recurrent infections, a need for hospitalization and the risk for the regeneration of autoreactive B cells (33,34).

4. T cell signal transduction

T cell proximal signal transduction pathways

T cell receptor first signal transduction. TCR possesses a dwarf intracellular domain, so the role of neighboring CD3 subunits is indispensable for signal transduction (35). When the T cells encounter their specific antigen presented in APC, the TCR interacts with the MHC molecule and antigen. This is followed by the binding of CD8/CD4 co-receptor δ 4 subunits to MHC1/MHCII β subunits, which prompt the TCR intracellular signaling pathway (36). Considering that none of the TCR integrates display autogenous kinase activity, non-receptor tyrosine kinases PTK are demanded.

The discovery of this PTK has enhanced the understanding of the TCR singling criterion. When co-receptors CD4/CD8 interact with MHC molecules, they facilitate the recruitment of key signaling kinases, Lck and Fyn, essential for downstream TCR activation (37,38). Lck and Fyn are members of the Sarcoma family kinases (SFK). SFK are non-receptor protein tyrosine kinases that are switched on by binding to various cellular receptors. They are primary kinases that render other protein tyrosine kinases active following their activation by receptor binding (39). Lck appears to be the crucial benefactor of TCR signal transduction (40). Yet, SFK members exhibit functional redundancy, rendering the assessment of Lck and Fyn's exact degree of contributions to TCR activation an unfathomable task (41).

Next, Src phosphorylates the immunoreceptor tyrosine-based activation motif (ITAM) of the CD3 ζ chain (42). Then, the phosphorylated ITAM recruits ζ associated protein (ZAP70) to bind to it. In turn, the ZAP70 protein becomes phosphorylated, which leads to its activation (40). The active ZAP70 phosphorylates the LAT (43,44). Subsequently, LAT protein activates Vav protein, which in turn signals the activation of the Rho/RAC GTPase pathway. RAC pathway mediates miscellaneous T cell function; for example, fostering actin organization (45).

In addition, Vav1 regulates the phosphorylation of receptors and tyrosine kinases through a negative feedback mechanism. LAT also musters and activates Src homology 2 (SH2)-domain-containing leukocyte protein of 76kDa (SLP-76) protein. The Activated SLP-76 derives the allelic exclusion of T cells (46). Furthermore, active SLP-76 binds to LAT through growth factor receptor-bound protein 2 (47). Finally, interleukin 2 inducible T cell kinase (ITK) binds to SLP-76. The association between the ITK and SLP-76 mediates several intracellular pathways, such as phospholipase C, RAS/RAF and MEK (48,49). All of these pathways conglomerate together and ultimately lead to the increased transcription of factors such as nuclear factor of activated T cells (NFAT) and activator protein 1, which promote T cell functions such as differentiation and cytokine release (49) (Fig. 3). In addition, recent breakthroughs have shown that LAT signaling is further regulated by phase separation. The process starts by the activation and recruitment of phospholipase C γ 1 (PLC γ 1)

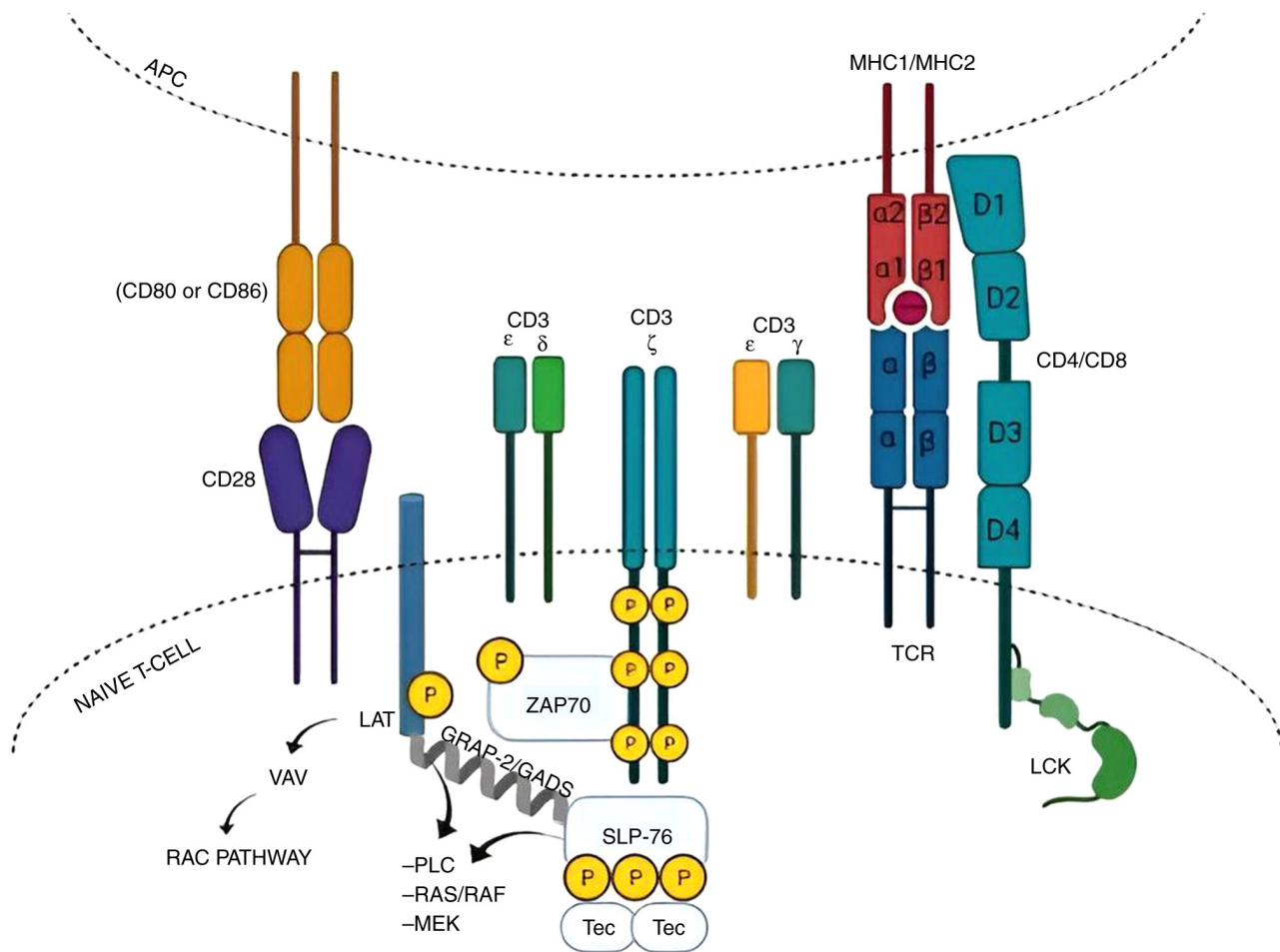


Figure 3. Proximal signal transduction. APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor; LAT, linker of activated T cells.

following TCR engagement with APCs. PLC γ 1 then facilitates phase separation of LAT by cross linking its molecules via SH2 domains, which eventually leads to the formation of phase-separated condensates. These condensates serve as a protector against tyrosine phosphatase, which prevents the dephosphorylation of LAT, which in turn helps sustain and amplify TCR signal transduction. In addition, the CD3 ϵ subunit of the TCR forms condensates with Lck to enhance phosphorylation. More importantly, phosphorylated CD3 ϵ recruits C-terminal Src kinase, a kinase that inhibits Lck function and dissolves concentrates, which makes this mechanism a natural on-off switch of the process (50,51).

Costimulatory signal transduction pathway. Costimulatory signals ensure the effectiveness of T cell activation. The central canon of T cell activation states that a paucity of costimulatory signals causes anergy or apoptosis (21). Among costimulatory receptors, CD28 is particularly crucial in enhancing TCR signaling and promoting robust immune responses, as recent studies demonstrate (52). As soon as CD28 binds to its ligand on the surface of APC, the intracellular production of phosphatidylinositol 3,4,5 triphosphate (PIP3) increases (53).

PIP3 aids the phosphorylation of Akt by PDK1 kinases. Next, Akt promotes the expression of NF- κ B. Therefore, it boosts Bcl-xl survival genes (54). Activated Akt lessens the activity of glycogen synthase kinase 3 to boot. GSK ameliorates

NFAT seclusion from the nucleus (55). It has been suggested that Akt also inhibits pivotal scaffolding protein essential for calcineurin access to the NFAT.

Hence, IL-2 production increases due to delayed nuclear export and calcineurin hindrance. In general, the CD28 amplifies T cell responses and cytokine production, serving as a key modulator of immune activation and longevity (56). This is particularly true when it comes to IL-2, which induces gene expression of antiapoptotic BCL-xL, decreases the time needed for naïve T-cell stimulation, increases cell adhesion and braces germinal center formation. Which explains why CD28 knockout mice showed faulty immune responses (57,58). The inhibition of the CD28 receptor may be used to treat autoimmune diseases and prevent allograft rejection and graft vs. host disease (59,60).

Second messengers inositol triphosphate (IP3) and diacylglycerol (DAG). Phospholipase C γ 1 represents a cardinal junction between proximal and distal transduction. Tec and SLP-76 phosphorylate and consequently activate PLC γ 1. Furthermore, Rlk which is another PTK, contributes to PLC γ 1 activation. The complete absence of Rlk and Tec in mouse models annihilates PLC γ 1 (61). PLC γ hydrolyzes membrane-bound phosphatidyl 4,5 bisphosphates (PIP2), generating IP3 and DAG at an equal ratio (62). IP3 activates

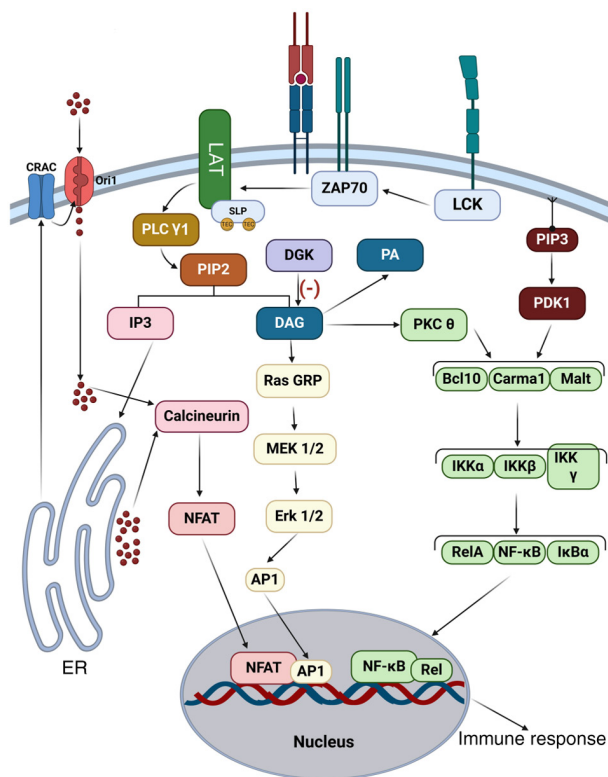


Figure 4. Second messenger and distal signal transduction. ER, endoplasmic reticulum; LAT, linker of activated T cells.

the IP₃-calcium-NFAT pathway. DAG collaborates with raised cytosolic calcium caused by IP₃ and directs protein kinase C to the membrane to be activated (Fig. 4) (63).

Distal signal transduction

IP₃-calcium-NFAT pathway. The IP₃ generated by PLCγ1 advances toward its receptors in the endoplasmic reticulum. This leads to a transient upswing of calcium (64). Intracellular calcium signaling is tightly regulated by ORAI1 channels, facilitating T cell activation and immune response modulation (65). Next, free calcium coheres to calcineurin and eventually activates it through phosphorylation. Thereupon, calcineurin dephosphorylates cytoplasmic NFAT by serine/threonine phosphatase enzyme (66). Calcineurin can be inhibited by cyclosporin and FK506 immunosuppressant drugs (67). The NFAT is then imported into the nucleus. Once inside, NFAT interacts with myriad transcription factors of the AP-1 (Fos/Jun) family protein, and DNA composite elements that accommodate their binding sites. Next, NFAT/Fos-Jun/DNA, highly stable triple amalgamates, regulate the transcription of variable inducible cytokine genes (68,69).

However, NFAT can solely bind to DNA in absence of AP-1, by a poorly understood mechanism. Once inside, NFAT interacts with myriad transcription factors of the AP-1 (Fos/Jun) protein family and DNA composite elements that accommodate their binding sites. Subsequently, NFAT/AP-1/DNA, a highly stable triple amalgamate, regulates the transcription of variable inducible cytokine genes. AP-1 is derived from DAG signaling. However, NFAT can solely bind to DNA in absence of AP-1, by a poorly understood mechanism (70).

Imbalances in calcium-calcineurin-NFAT signaling can lead to T cell anergy, regulated in part by E3 ubiquitin ligase and its downstream pathways. The dysregulation of NFAT activation and import or impaired calcium signaling, which are due to ORAI1 mutation causing aberrant calcineurin-NFAT activation, bring about severe combined immunodeficiency syndrome (71). This can be managed partially by lithium chloride, as it thwarts the nuclear export of NFAT (72).

DAG-kinase pathways. Membrane-bound DAG represents the second generated molecule of PIP₂ through PLCγ1 hydrolysis (62). DAG recruits Ras-guanyl releasing protein 1 (Ras-GRP1) and activates Ras by converting GDP to GTP (73). Active Ras-GRP1 consequently activates the RAF1-MEK-ERK pathway, leading to increased cytokine release and cell proliferation (74).

In addition, Ras-GRP1 supports the development of αβ and γδ cell lineages that secrete IL-17 and increase CD8 cell expansion (75,76). SLE patient and mouse models have exhibited an abnormal expression of Ras and Ras-GRP1, revealing a link between this pathway and SLE pathogenesis (77).

The other mission for DAG is accomplished by the activation of protein kinase (PK)Cθ by attracting it to the immune synapse and then phosphorylating it (78). Consequently, active PKC mediates the TCR-dependent activation of NF-κB (79). NF-κB signaling plays a pivotal role in T cell differentiation, regulating IL-2 production and balancing Th cell subsets (80). The impairment of these pathways results in various diseases, such as lymphoma, autoimmune diseases and flawed T cell activation (81,82). DAG and its downstream metabolites are essential for T cell function, with DAG kinase ζ acting as a key modulator in this signaling cascade. At the same time, DGK kinase ζ converts DAG to phosphatidic acid in numerous lymphoid tissues, particularly within T cell compartments (83).

5. Conclusion

T-cell research is rapidly advancing, unveiling groundbreaking therapeutic applications such as CAR-T cell therapy for autoimmune diseases and novel cancer immunotherapies. Despite these strides, several challenges remain. A key limitation of current knowledge is the incomplete understanding of long-term T-cell fate, particularly in the context of engineered therapies. Additionally, the intricate role of T-cell metabolism in immune regulation is still not fully elucidated, and comprehensive clinical data on emerging treatments remain limited. Another pressing area of investigation is the phenomenon of T-cell exhaustion, where prolonged activation leads to diminished immune responses. Deciphering the precise mechanisms underlying exhaustion and fine-tuning signaling pathways are crucial steps toward optimizing therapeutic strategies.

Moving forward, future research should prioritize enhancing CAR-T cell persistence to improve long-term efficacy, unraveling the metabolic influences that govern T-cell function, and developing next-generation immune checkpoint modulators to fine-tune immune responses. These efforts will drive the evolution of personalized immunotherapies, offering more precise and durable treatment options for immune-mediated diseases and cancer. As our understanding of T-cell regulation continues to expand, new opportunities will emerge

to harness the immune system more effectively, striking a delicate balance between robust defense and immune tolerance. Ultimately, these advancements will shape the future of immunotherapy, transforming patient outcomes and redefining modern medicine.

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Authors' contributions

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Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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