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# The Role of LCK Kinase in T-cell Antigen Receptor Signaling

Role kinázy LCK v antigenní signalizaci T-lymfocytů

Bachelor's thesis

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**Poděkování:**

Zejména bych chtěl poděkovat svému školiteli Mgr. Ondřeji Štěpánkovi Ph.D. za odborné vedení mé práce, neskonalou trpělivost a ochotu při poskytování cenných rad. Současně bych rád srdečně vyjádřil svůj vděk za možnost zapojit se do tématem příbuzného projektu a osvojit si některé metody a zásady laboratorní praxe. Za veškerou podporu bych rád poděkoval celé své rodině a v neposlední řadě svým přátelům.

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V Praze, 13. 8. 2020

# Abstract

LCK activity is crucial for the triggering of the entire T cell activation process. The primary function of LCK is to convert the signal of TCR:pMHC ligation into the intracellular environment. The outcome of the LCK-triggered pathway is T cell activation, cytokine production, differentiation, and clonal expansion. This thesis provides a summary of recent knowledge about the unique position of LCK in the T cell signaling machinery as well as an overview of molecules and interacting partners that regulate LCK activity. It describes the importance of the LCK-coreceptor association for optimal TCR signaling and physiological thymocyte development and mentions discussed adaptor role of LCK in the T cells.



**Keywords:** LCK, T-cell, antigen, kinase, enzyme



# Abstrakt

Aktivita LCK kinázy je nezbytně nutná pro zahájení procesu aktivace T lymfocytu. Primární funkcí LCK je převod informace o vazbě pMHC glykoproteinů na TCR do vnitřního prostředí buňky. Výsledkem kaskády, jež aktivní LCK spouští, je aktivace T buňky, produkce cytokinů, diferenciace a klonální expanze. Tato práce poskytuje souhrn současných znalostí o LCK kináze a její nenahraditelné roli v TCR signalizaci a stejně tak přehled nejvýznamnějších regulátorů a interakčních molekul. Dále pak popisuje význam interakce LCK s koreceptory pro optimální TCR signalizaci a fyziologický vývoj thymocytů a též zmiňuje diskutovanou roli LCK jako adaptorového proteinu T buněk.



**Klíčová slova:** LCK, T lymfocyt, antigen, kináza, enzym

# List of abbreviations

| | |
|---|---|
| TCR | T Cell Receptor |
| MHC | Major Histocompatibility Complex |
| APC | Antigen Presenting Cell |
| LCK | Lymphoid Cell Kinase |
| SFK | Src-family Kinase |
| ZAP-70 | Zeta-chain Associated Protein kinase 70 |
| LAT | Linker of Activation of T cells |
| NFAT | Nucleolar Factor of Activated T cells |
| SH | Src-homology |
| KO | Knock-Out |
| KI | Knock-In |
| KD | Knock-Down |
| WT | Wild Type |
| ITAMs | Immunoreceptor Tyrosine-based Activation Motifs |
| cDNA | complementary DNA |
| DP | Double-Positive |
| PLCγ1/2 | Phospholipase C γ 1/2 |
| mTOR | mammalian Target of Rapamycin |
| NK | Natural Killer |
| KIRs | Killer Inhibitory Receptors |
| PTP | Protein Tyrosine Phosphatase |
| DPho-LCK | Doubly-Phosphorylated LCK |
| PI-3K | Phosphatidyl Inositol-3 – Kinase |
| IP3 | Inositol-3 – Phosphate |
| DAG | Diacylglycerol |
| OVA | Ovalbumin peptide |
| OT-1 | Ovalbumin reactive TCR |
| Treg | Regulatory T cell |
| CSK | C-terminal Src Kinase |
| PKC | Protein Kinase C |

| | |
|---|---|
| kDa | kiloDalton |
| ICOS | Inducible T cell COStimulator |
| MAPK | Mitogen Activated Protein Kinase |
| GRB2 | Growth factor Receptor Bound protein 2 |
| SLP-76 (LCP 2) | Lymphocyte Cytosolic Protein |
| SOS | „Son of Sevenless“ |
| ERK | Extracelular signal Regulated Kinase |
| AKT (PKB) | Protein Kinase B |
| NF-κB | Nuclear Factor kappa-light-chain-enhancer of activated B cells |
| SHP-1 | Src-Homology Phosphatase 1 |
| JKAP | JNK-pathway Associated Phosphatase |
| FcγR | Fcγ Receptor |
| PAG | Phosphoprotein Associated with Glycosphingolipid-enriched microdomains |
| mRNA | messenger RNA |
| Hnrpll | Heterogeneous Nuclear Ribonucleoprotein L Like |
| PTPRC | Protein Tyrosine Phosphatase Receptor Type C |
| THEMIS | Thymocyte-expressed molecule involved in selection |
| CD | Cluster of Differentiation |
| HSP | Heat-Shock Protein |
| Val | Valin |
| Ser | Serin |
| Ala | Alanin |
| CBL | E3 Ubiquitin-Protein Ligase |
| TSAd | T cell Specific Adaptor Protein |
| HCK | Hematopietic Cell Kinase |
| FGR | Tyrosine-protein Kinase FGR |
| LYN | Tyrosine-protein Kinase LYN |
| FYN | Tyrosine-protein Kinase FYN |
| GADS (GRAP2) | GRB2 Related Adaptor Protein 2 |
| ARS | Activation-Responsive Sequence |

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# 1 Introduction

The healthy immune system provides an efficient recognition of foreign structures while keeping a tolerance against self-antigens. Both foreign and self-antigens are presented by antigen-presenting cells on the MHC glycoproteins class II and I, respectively. The specific discrimination between the foreign and self-antigens is driven by the adaptive immune system, particularly T cells. The critical receptor in this process is the T-cell receptor (TCR), which binds to peptide-loaded MHC molecules. T cell development includes positive and negative selection. During the positive selection only the clones, whose TCR can bind the MHC glycoproteins, get the survival signal. The clones that are unable of signaling through the TCR start apoptosis. During the negative selection, only the clones that do not respond to self-antigens pass through. This double-step selection during the development in the thymus creates mature either CD8+ or CD4+ T cells that recognize antigen fragments presented on MHC class I or II, respectively. However, the signal propagation following after TCR triggering is carried by LCK. Lymphoid cell kinase is a cytosolic protein that temporarily interacts with the TCR complex after its stimulation. This interaction and following phosphorylation of the TCR/CD3 complex enables to convert an extracellular antigen-binding event into a biochemical signal within the intracellular environment. The subsequent signaling pathways lead to activation of effector molecules, production of secondary messengers, or regulation of gene expression in the nucleus. The result of these events within the intracellular environment is activation, cytokine production, differentiation, and proliferation of the T cell. Thus, LCK has an irreplaceable role in proximal signal propagation that ensures the normal development and function of the adaptive immune system.

This thesis aimed to demonstrate the importance of LCK in T cell development and proximal signaling that triggers T cell activation. The aim is to review our current knowledge of the role of LCK in TCR signaling and its regulation. The main aspects of the thesis are LCK domain organization, catalytic but also a nonenzymatic function of LCK, conformational changes, that relate to different LCK activity, and the effect of LCK-coreceptor association in T cells and developing thymocytes.

# 2 Structure of the Lymphoid cell kinase LCK

LCK belongs to the Src family of tyrosine kinases (SFKs)[1]. SFKs comprise proteins that generally have a conserved structure. However, they differ in distribution within the hematopoietic subpopulations. Myeloid cells primarily express such kinases as HCK, FGR, and LYN. On the other hand, leukocytes such as B cells primarily express LYN, FYN, and BLK, while T cells LCK and FYN-T[1].

LCK has a relative molecular mass of 56 kDa[2]. LCK's N terminus is modified, which creates a hydrophobic anchor that provides the interaction of LCK with the membrane sheet[1] (Figure 1). The fatty acid modifications are essential for a specific localization in a T cell and restrict LCK presence mainly for the plasma membrane[3]. Due to the palmitates on cysteines number 3 and 5[4] and myristates on glycine number 2[5], LCK can be anchored to a specialized membrane compartment called detergent-resistant microdomains. Src family kinases lacking myristoylation and palmitoylation have lost the capability to participate in TCR signaling, due to the inability to interact with this membrane microdomains[6]. Such LCK behaves as soluble instead of membrane-bound[5]. However, the Jurkat T cell line that expressed LCK with cysteines number 3 and 5 substituted to serines was generated[7]. Surprisingly, the LCK mutant did not completely lose the ability of membrane localization[7]. The fact, that these LCK mutants could partially localize the membrane suggests the role of coreceptor in LCK recruitment.

The coreceptor binding domain within the LCK is located at the N-terminus (Figure 1). It enables the coupling with either CD8 or CD4 coreceptors via a dicysteine motif[8]. Coreceptors are surficial molecules expressed in T cells and function in the recruitment of LCK to the proximity of triggered TCR[9]. In mature peripheral T cells, two types of coreceptors can be distinguished – CD4 or CD8. They are responsible for recognition of either peptide fragments complexed with MHC class I (endogenous peptides) or MHC class II (foreign antigen fragments), respectively[10]. LCK possesses cysteine residues 20 and 23, which are responsible for the interaction with the coreceptor. A published data indicates that the LCK interacting domain is 12 amino acid long in the case of CD4 and around 30 amino acid in CD8α, respectively[9].

The own kinase domain follows the coreceptor interacting domain. The other regions are referred to as Src-homology domains. The first one, SH2 domain, comprises 100-amino acid and forms an antiparallel β-sheet of 5 strands - βa-βe. The SH3 domain is 60 amino acid long and consists of a central β-sheet flanked by two α-helixes A and B[11]. The C terminal tail is an

essential site of phosphorylation, which determines the molecule conformation and activity[2] (Figure 1).

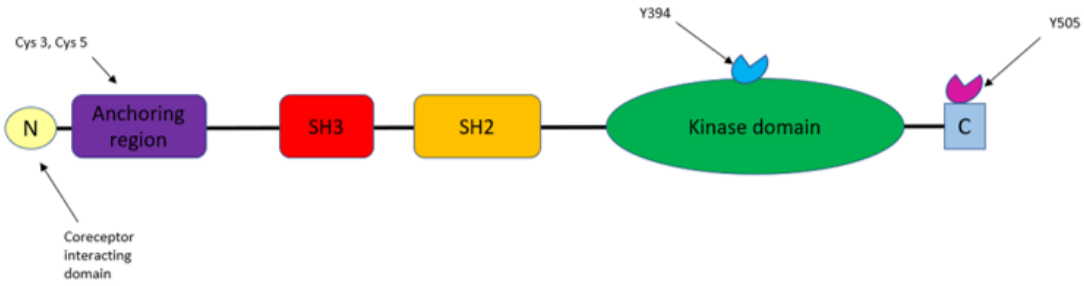


***FIGURE 1*** *- LCK domain organization: N terminus of the LCK molecule carries the cysteine residues that are responsible for the interaction with a coreceptor and the specific sites of the fatty acid modification, that enables the membrane attachment. The membrane anchoring region is followed by the SH3 and SH2 domains, that function in intermolecular interactions as well as in LCK activity regulation. The catalytic domain is located on the C terminus and possesses the activation tyrosine 394. The C-terminal tail of the LCK contains the inhibitory tyrosine number 505.*

# 3 The role of LCK in TCR signal transduction process

## 3.1 LCK - the most proximal member of TCR signaling cascade

LCK has the most proximal role in T cell signaling initiation after the TCR and therefore is crucial for proper positive and negative selection in T cell development[12]. Straus and Weiss have shown the importance of LCK in the experiments with JCaM1 Jurkat T cells, which are LCK-knock out and thus not responsive to a TCR stimulation [12].

LCK works within the membrane area, where the T cell interacts with an antigen-presenting cell (APC). The region of interaction is called the immune synapse[13]. The APCs possess increased expression of major histocompatibility complex (MHC) molecules on its surface[13]. The MHCs are classified as class I or II presenting a fragment of endogenous or a foreign peptide (pMHC), respectively[10] (Figure 2). When the T cell recognizes a relevant pMHC via its TCR, LCK is brought to the center of the synapse, where it interacts with CD3 complex and phosphorylates ITAM residues within it[14] (Figure 2). The ITAM regions generally include two separated motifs consisted of 4 amino acid sequence YXXL[15].

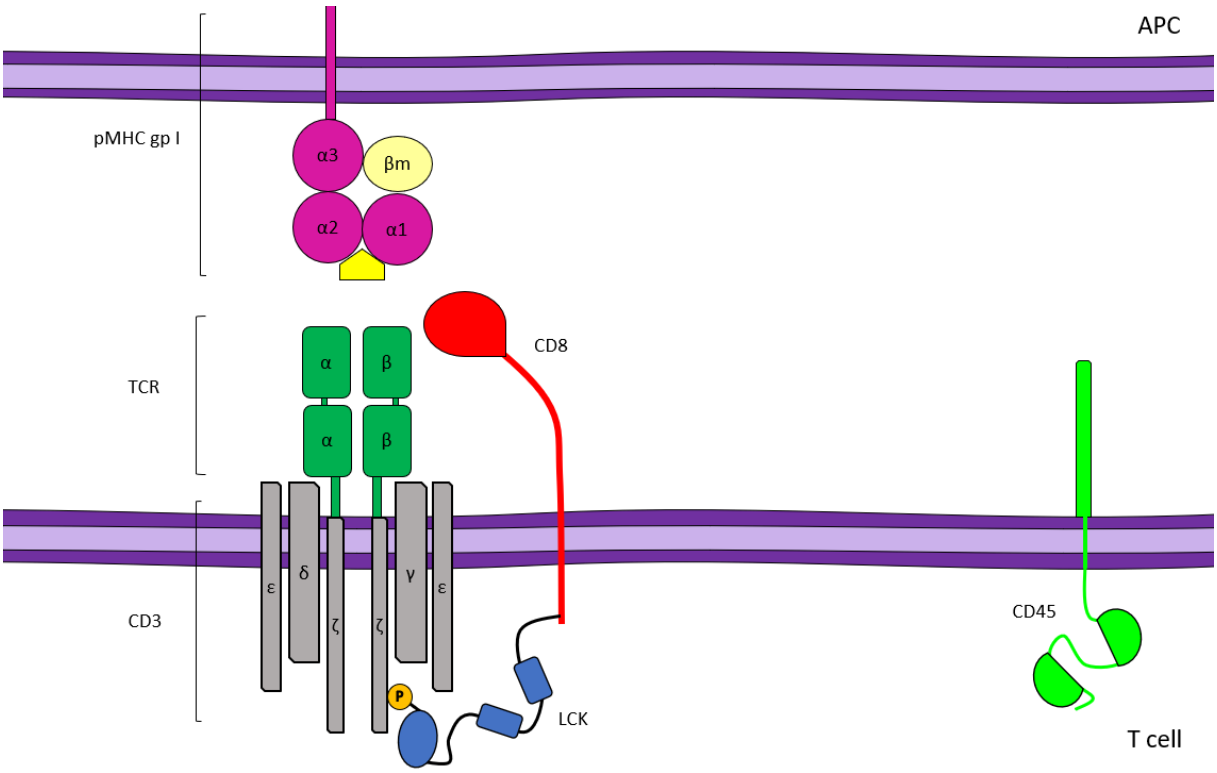


***FIGURE 1*** *– The immune synapse: The scheme demonstrates the membrane area of the interaction between an APC and a CD8+ T cell. The APC is presenting a fragment of a self-antigen on surficial molecules referred to as MHC glycoproteins class I. The T cell on the other side recognizes the peptide fragment presented on the MHC class I via antigen-specific T cell receptor (TCR). TCR is associated with the CD3 complex, which can be phosphorylated on its intracellular domains. After the antigen*

*binding, the TCR changes its conformation and recruits LCK N-terminally associated with the CD8 coreceptor. CD45 protein tyrosine phosphatase is excluded from the center of the immune synapse. Active LCK phosphorylates the ITAMs within the CD3 complex. The ITAMs phosphorylation triggers the TCR downstream signaling and allows the recruitment of other signaling molecules.*

A recent study revealed a possible mechanism of the interaction of LCK with stimulated TCR[16]. CD3ε contains a unique motif referred to as RK, which is responsible for the binding of the SH3 domain of LCK. When the TCR binds the ligand, the RK motif becomes accessible for the SH3 domain of LCK, which can bind and phosphorylate the ITAMs within the CD3 complex[16].

A mutated line of Jurkat T cells, referred to as JCaM1, provides genetic evidence about the indispensable role of LCK in T cell activation and thymocyte development. JCaM1 mutant lacks LCK kinase activity, and thus no CD3 phosphorylation can be observed in these cells. However, the phosphorylation of the CD3 complex was restored with the transfection of cDNA of wild-type (WT) LCK[12], which fact confirms the significant involvement of LCK in CD3 phosphorylation.

## 3.2 ZAP-70 recruitment

Double phosphorylation of the ITAMs within the intracellular part of the CD3 complex allows the recruitment and binding of another protein tyrosine kinase ZAP-70 and disruption of its autoinhibited state[17]. The docking of ZAP-70 onto the ITAMs is held via interaction with the SH2 domain of ZAP-70[18]. ZAP-70 requires phosphorylation of its interdomain on Y319 and Y315 to become fully active[19]. In an unphosphorylated state, the SH2 modules of ZAP-70 create a pair of clamps on the kinase domain[19]. The phosphorylation, provided by LCK interacting with ZAP-70 via its SH2 domain[20], results in a conformational change and releasing of the kinase domain from the SH2 clamps[19]. A point mutation on Y319 of ZAP-70 resulted in lowered both positive and negative selection in ZAP-70-Y315F (Tyrosine 315 substituted for Fenylalanin) knock-in mice[20]. Another site of LCK-dependent phosphorylation appeared to be Y494 within the catalytic domain of ZAP-70[18]. Thus, multisite phosphorylation by LCK is necessary for ZAP-70 to sustain in an active conformation[18].

## 3.3 Signaling downstream ZAP-70 leading to T cell activation

As ZAP-70 is activated, it can phosphorylate the linker of activation of T cells (LAT)[14]. ZAP-70 and LAT interact through the “bridging” of the SH3 domain of ZAP-70 and a proline-rich motif within LAT[21].

LAT possesses 9 different regulatory tyrosines, which triggers various signaling pathways when phosphorylated[17]. Phosphorylation of these tyrosines is responsible for the recruitment of phospholipase C1 (PLCγ1)[14], which is involved in calcium-dependent Ras/MAPK activation. Other signaling molecules activated by LAT phosphorylation are AKT/mTOR[17], GRB2, and GADS, that can bind SOS and SLP-76 molecules, and activate downstream effectors such as Ras, Rac and Rho GTPase[14].

When LAT is phosphorylated on Y132, it can bridge the SH2N domain of PLCγ1 and activate it[22]. PLCγ1 activity leads to the production of secondary messengers – diacylglycerol (DAG) and inositol-3-phosphate (IP3)[12]. DAG functions near the membrane and activates protein kinase C (PKC), which is essential for activation of RasGRP-dependent signaling pathways[23],[24]. IP3 is crucial for the controlled releasing of $Ca^{2+}$ from the vesicles of the endoplasmic reticulum[12]. The cytosolic calcium influx creates a signal for the activation of many transcription factors such as NF-κB and expression of the Nucleolar factor of activated T cells (NFAT). Increased NFAT expression can also be induced directly by AKT-dependent NF-κB activation[25].

Another crucial kinase called the mammalian target of rapamycin (mTOR) is involved in T cell activation after the recruitment of PI-3K in the presence of costimulatory receptor (CD28, ICOS)[26]. Its activity leads to changes in overall metabolism, actin organization, growth, and apoptosis, that drive the T cell towards the activation[26]. The functions of mTOR were abrogated after inhibition of PI-3K or AKT supporting the upstream character of the molecules. The CD28 signaling is essential for mTOR function. However, even the presence of CD28 cannot suppress the effect of mTOR inhibition[26]. These cell responses were described as anergic after the TCR stimulation. Moreover, the mTOR-AKT-PI-3K axis seems to regulate the expression of FoxP3 negatively, and thus, TCR stimulation in the absence of mTOR results in differentiation of regulatory instead of activated effector T cells[27].

## 3.4 LCK possesses a specialized function in cytotoxic T cells and NK cells

LCK can serve as an initiator of signaling pathways in NK cells and provide an FcγR-dependent activation[28]. This function is specific strictly for LCK among the SFKs. It is coupled with an FcγR, after which stimulation LCK phosphorylates its substrates, which means ZAP-70 and SYK. The results of the overall FcγR signaling are similar to TCR signaling - activation of PLCγ2 and the production of secondary messengers[28].

Except for the activating function, LCK can trigger a series of cell responses that generally have opposite results[29]. In NK cells, LCK is also coupled to a killer inhibitory receptors (KIRs). These KIRs, when phosphorylated by LCK, are responsible for the suppression of the activity of NK cells and inhibition of FcγR-mediated cytotoxicity in T cells. When an MHC class I engages the KIR, LCK is activated and phosphorylates the receptor. The phosphorylated KIR suddenly recruits cytosolic associated Src-homology phosphatase (SHP1), which dephosphorylates the proximal PTKs connected with the FcγR pathway and counters the activation of cytotoxicity[29]. Moreover, when LCK phosphorylates the KIR, it enables the recruitment of p85α subunit of PI-3K. The outcome of the KIR signaling includes a PI-3K-mediated activation of antiapoptotic serine/threonine kinase AKT[30].

# 4 Regulation of LCK activity in T cells

LCK activity is known to be regulated via phosphorylation of mainly two functionally critical amino acids, specifically inhibitory tyrosin 505 within C-terminus tail and then tyrosin 394 inside of an activation loop[31]. The mechanism and site of phosphorylation are relatively conserved among the Src-family kinases. Autophosphorylation on the Y394 brings catalytic activity to the LCK[31]. On the other hand, phosphorylation of the inhibitory C-terminal tail on tyrosine residue number 505 leads to the stabilization of an autoinhibited, closed conformation of LCK[32]. The phosphorylation on Y505 provides another Src-family kinase CSK, which activity downregulates LCK[33] (Figure 3).

This mechanism of the inhibitory enclosing of the LCK molecule is held via an intramolecular interaction of the C terminus with regulatory domains. These are referred to as SH2 and SH3 domains[11].

Palacios and Weiss published a transient state of LCK conformation. It was referred to as primed LCK[1], which does not adopt the closed conformation, but still is not activated yet. It is dephosphorylated on inhibitory Y505 already but lacks the modification on Y394 to become activated[1] (Figure 3).

A recent study using an LCK biosensor supported that LCK needs to be "de novo" phosphorylated on its activation loop, while the only dephosphorylation on the inhibitory C terminus is not sufficient for its activation[34].

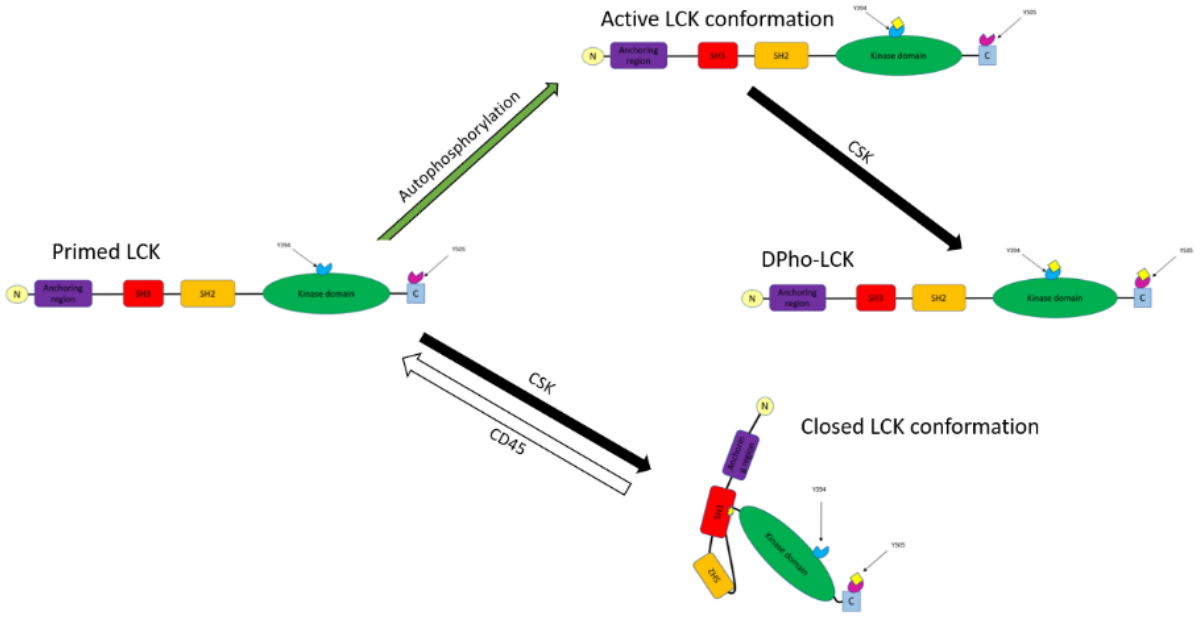

***FIGURE 2*** *- LCK conformations: Generally, LCK exists in two structural conformations within a T cell. It is either an open or closed conformation. However, the open conformation includes three forms of LCK that differ in phosphorylation of regulatory tyrosines and catalytic activity. The initial state is referred to as Primer LCK, and it is not phosphorylated on neither activating nor inhibitory tyrosine. However, it does not have a catalytic activity. The primed LCK becomes active after an autophosphorylation of its activating Y394 (indicated by the presence the yellow square as phosphate). The activity of CSK kinase converts the primed LCK molecules into the closed, inactive form. CSK phosphorylates the C-terminal inhibitory Y505. The Y505 phosphorylation causes an enclosing of the LCK molecule via an intramolecular interaction of the C-terminus with the SH3 domain. Such LCK conformation suppresses the catalytic activity. The autoinhibited conformation of LCK can be disrupted by the activity of CD45, that dephosphorylates the C-terminal tyrosine and releases the intramolecular interaction within LCK. The last conformation of LCK is referred to as DPho-LCK (doubly phosphorylated), because it possesses both Y394 and Y505 phosphorylated. Although the inhibitory tyrosine is phosphorylated as well, the DPho-LCK has a kinase activity comparable to LCK in the active conformation (pY394).*

In addition to the conventional open, closed, and primed form, a DPho-LCK (doubly phosphorylated LCK) was also described[35] (Figure 3). It adopts an open conformation, while phosphorylated on both Y505 as well as Y394 (pY505-pY394-LCK) residues at the same time[35]. Its kinase activity seems to be comparable to pY394-LCK. The authors mention that DPho-LCK could be derived from pY394-Y505-LCK by the activity of CSK[35].

## 4.1 Existence of preactivated LCK pool

It was thought that the activation loop of LCK becomes phosphorylated after TCR triggering only, and thus the phosphorylation is present only in stimulated cells[12]. Nevertheless, Nika et al. proposed that resting T cells and thymocytes do express high amounts (nearly 40 % of all LCK count) of LCK pool containing pY394[35]. Removal of approximately 90% of preactivated LCK resulted in a retardation of further CD3 ITAM phosphorylation, so it was assumed, that constitutively activated LCK acts as an essential initiator, which shortens the delay from ligand binding to ITAM phosphorylation and enhances the sensitivity of T receptor signaling[35]. However, there are some contradictory findings, for example, that the pool of pY394-LCK does not make nearly a half of the LCK amount, but only around 2 %[36]. The authors argued that Experiments by Nika were probably influenced by phosphorylation of LCK after T cell lysis without adding a tyrosine kinase inhibitor, so the result with approximately 40 % of LCK preactivated could be an artifact[36].

## 4.2 CD45 signaling gatekeeper and it is opposing functions

LCK must be primarily dephosphorylated by a critical regulator CD45 protein tyrosine phosphatase (PTP) to become active[37]. CD45 was referred to as a signaling gatekeeper because it is highly responsible for maintaining of preactivated LCK pool[38]. The amount of preactivated LCK was published to be essential for initiating of the TCR signaling events and for their further modulating and exclusion between weak and strong TCR signals[38]. CD45 has an essential role in reversing the inhibitory effect of CSK on LCK. It was observed that in $CD45^{-/-}$ deficient

cells, the C-terminus of LCK is hyperphosphorylated, and thus LCK is maintained in the autoinhibited state[39].

The major outcome of CD45 activity is definitively a TCR signaling stimulation. Nevertheless, CD45 has multiple functions within a T cell. It can dephosphorylate either pY394 and turn the LCK into the primed/inactive form[36], or catalyze the dephosphorylation of ITAMs in the TCR/CD3 complex to terminate the TCR signal transduction[38]. This mechanism prevents undesirable continuous signaling and refreshes the TCR signaling capability. For this reason, CD45 is excluded from the synapse between APC and T cell to the periphery[40]. The segregation of CD45 involves the equilibrium between LCK and phosphatase CD45, which results in increased activation of LCK molecules[41].

CD45 ectodomain seems to drive the exclusion of CD45 from the close contact between an APC and a T cell[42]. It was published to be allowed because of the mechanical properties of the ectodomain, like increased rigidity due to the higher presence of cysteines forming disulfide bonds[41].

However, CD45 seems to have a primary role in TCR signaling regulation processes, another PTPs, which can partially substitute CD45 when not functioning, do probably participate[38]. Taken together, CD45 is definitively essential for proper T receptor signal transduction, so there must be a complex regulatory system of its activity.

CD45 interacts with the Y192 within the SH2 domain of LCK[43]. A mutation of this residue led to a rapid disruption of TCR signaling with a phenotype reminding the CD45 deficiency and so resulted in the C-terminal hyperphosphorylation of LCK. Because of that, the LCK active pool decreased. It suggests the conclusion that LCK with Y192 phosphorylated or somehow modified is not an available substrate for dephosphorylation on the inhibitory Y505 provided by CD45, which would bring LCK the catalytic activity[44].

The CD45 extracellular domain is highly glycosylated, and it creates several CD45 isoforms caused by alternative splicing of its transcript[42]. These isotypes are differently expressed in T cells, depending on their activation status and developmental stage[45]. CD45 mRNA was described as containing 34 exons, whereas numbers 4, 5, and 6 of the *PTPRC* (Protein tyrosine phosphatase receptor type C) gene[41] undergo alternative splicing, either by activating of cis- or trans-acting factors, into eight isoforms[37]. Six of these isoforms were reported to be expressed

in human hematopoietic cells[46]. However, there is evidence that exon number 7 can also be involved in the process[47].

The alternative splicing of the exons 4, 5, and 6 creates isotypes referred to as CD45RO, CD45RA, CD45RB, and CD45RC[48]. The activated or memory T cell highly express the shortest isoform – CD45RO, while naive T cells show an increased expression of longer CD45RA/B isoforms[49]. It was supported by an experiment, where naive $CD45RA^+$ Treg cells interrupted the expression of CD45RA immediately after activation and became $CD45RO^+$ memory regulatory T cells (Tregs)[50]. The alternative splicing is initiated by activation of such pathways as Ras and PKC after TCR stimulation[51]. These CD45 isoforms do homodimerize. Most importantly, the shortest isoform – CD45RO has the highest efficiency of dimerization. The rate of homodimerization directly corresponds with the inhibited function of the CD45 isotype. Thus CD45RO is the least competent to activate LCK[52]. Memory cells expressing this isotype are less sensitive to a TCR stimulation, and the overall tyrosine phosphorylation is decreased, as well as the phosphorylation of ZAP-70 and production of IP3 compare to CD45RA/B+ naive T cells[53]. Some publication indicated that the expression of various CD45 isoforms can provide the ability to interact with another signaling molecules[54]

If the shortest isoform CD45RO is the least competent to induce LCK activation, what makes the memory T cell responses such robust and rapid? Xu and Weiss provided possible explanations of this phenomenon. The efficient and robust responses of CD45RO+ memory T cells can potentially be caused by other factors than by a simple increase in TCR signaling magnitude[52]. A CD45-independent signalization in these cells was not excluded as well[52], however, probably the most important mechanism of how memory T cells respond more efficiently is that the CD45RO associates with CD4 coreceptor more likely than the other isoforms[55].

The existence of alternative splicing instead of the expression of all CD45 isoforms simultaneously was reasoned by Xu and Weiss. Because the CD45 molecule is relatively large and already highly present in the plasma membrane, the expression of all these several isotypes simultaneously could create a physical blockade of the interactions with other cells including adhesion and antigen presentation provided by APCs[52].

The CD45 isoform expression also affects the migration of Tregs within tissues. $CD45RO^+$ Tregs shown to have a much higher ability of skin-homing than $CD45RA^{+}$[50].

Increased expression of heterogeneous nuclear ribonucleoprotein L-like *(Hnrpll)* determines a specific silencing of the exons within PTPRC, and thus the preferential creation of CD45RO in memory T cells. When the *Hnrpll* carries a point substitution of Thymin to Adenin, which leads to change in codon from $Val_{136}$ to Ala, it creates a phenotype referred to as a *thunder*. The *thunder* mice have lost the ability of gene-dosage-sensitive silencing of exons within the *PTPRC* gene containing the ARS splicing-silencer motif, and these memory T cells do not express CD45RO preferentially[56].

As was already mentioned, except CD45, another PTPs takes place in the regulation of LCK activity. CD148 is also involved in TCR signaling regulation[57]. It even seems to have very similar properties and functions in T cells. Activation of CD45 deficient T cells was observed in the presence of CD148. The effect is suggesting some redundancy in the function of CD45 and CD148[58]. In the opposing situation, in CD45 WT T cells, the activating preference is exceeded by the capability of CD148 to dephosphorylate the activation loop of LCK and thus inhibit it[58]. The results suggest the involvement of CD148 in the regulation of LCK activity and some level of coordination and redundancy with CD45.

Src-homology phosphatase 1 (SHP1) is another PTP with a function similar to CD45 and CD148[58] – to inactivate LCK by dephosphorylating of its pY394. SHP1 works as an LCK inhibitor[59] and is crucial for proper TCR signaling and development of single-positive thymocytes[60]. However, SHP-1 activity does not lead to the displacement of the closed conformation of the LCK. It cannot activate LCK by dephosphorylating on the pY505 such as CD45 does[59]. The loss of SHP-1 causes hypersensitivity and impairs the T cell selection thresholds[59]. SHP-1 can also have an inhibitory effect downstream the TCR - it is a negative regulator of PI-3K activity[61].

One more PTP, that provides the LCK inhibition is JKAP[62]. JKAP directly dephosphorylates LCK on pY394 and attenuates TCR signaling[62]. JKAP-knock-out mice shown only reduced antibody production in reaction to an immunization. However, it was producing autoantibodies as well. Thus, JKAP deficient mice are more likely to develop autoimmunity. Interestingly, while in T cells, JKAP has negative regulatory functions on TCR signaling, in B cells, its function is stimulatory[62].

## 4.3 Regulation of LCK activity via negative feedback of downstream signaling molecules

Another possible way, how LCK activity can be regulated is via negative feedback of downstream signaling molecules. After the inhibition of ZAP-70, a substrate of LCK, a rapid decrease of SLP-76 and LAT (ZAP-70 downstream molecules) phosphorylation was observed[10]. Nevertheless, ZAP-70 inhibition caused enhanced phosphorylation of upstream signaling molecules, most importantly, LCK[43].

Additionally, increased phosphorylation of Y493 within ZAP-70 appeared in SLP-76 and LAT deficient Jurkat T cells. The effect suggests a conclusion that the increased ZAP-70 phosphorylation was caused by increased LCK activity as a negative feedback mechanism[43].

One more site of phosphorylation possibly carries an essential regulatory role within the LCK. Serine on position 59 (S59) is located within the N-terminal domain of LCK and can be phosphorylated by MAPK extracellular signal-regulated kinase (ERK). The phosphorylation of S59 appears when the associated TCR is stimulated by a strong agonist. This S59 phosphorylation should be responsible for a change in SH2 domain binding specificity. It seems that the modification results in a blockage of SHP1 recruitment. The blockage of SHP-1 recruitment creates another mechanism of antigen discrimination, where weak agonists would not trigger responses leading to SHP1 recruitment blockage, while the strong ones would suppress the potential inactivation of LCK kinase activity[63].

However, calcineurin - a calcium-calmodulin dependent serine/tyrosine phosphatase can also dephosphorylate LCK on S59. No change in LCK phosphorylation was observed after inhibition of calcineurin, while the LCK downstream molecules such as ZAP-70, LAT, and SLP-76 appeared hyperphosphorylated. So S59 phosphorylation probably does not directly affect LCK activity or even degradation in the proteasome[64] but impairs the signal transduction due to the enhanced ability of LCK to connect with its downstream molecules[10].

A more recent publication has revealed that SHP-1 associated with a protein referred to as THEMIS in developing thymocytes as well as in mature T cells[65]. The THEMIS-SHP-1 association is dependent on Grb2 that serves as a bridge. Moreover, the complex of THEMIS-Grb2-SHP-1 allows the interaction with phosphorylated LAT. Surprisingly, the THEMIS-Grb2-SHP-1 was not associating with LCK during the observations. They generated THEMIS-knock down-mice and found out that however, the ITAM phosphorylation was significantly increased, the LCK activity in the mean of Y394 phosphorylation remained unaffected. Full

knock-out of THEMIS protein possessed an increased CD3 and ERK phosphorylation, CD69 (marker of activated T cells) expression, and enhanced TCR signaling that resulted in a higher rate of activation-induced apoptosis, however, the LCK phosphorylation was unchanged. Finally, the other mice generated were LCK knock-in with Ser59 substituted to Ala. Such mice did not possess any pathological phenotype - its TCR repertoire, coreceptor, and CD3 expression and cytokine production was comparable to the WT as well as the thymus and lymph nodes size and composition[65]. These results are not completely consistent with the data published by Stefanova et al., that support the model of S59 dependent SHP-1 recruitment blockage. However, Paster et al. support that THEMIS-SHP-1 association essentially influences the TCR signaling by a negative feedback mechanism, they did not observe any THEMIS-SHP-1-LCK association.

## 4.4 Proteasomal degradation of active LCK molecules

The binding of LCK to the HSP90-CDC37 complex also seems to be an essential regulatory mechanism. When LCK binds with the HSP90-CDC37, it results in protection from degradation of the LCK in the proteasome[35]. Nevertheless, the LCK stabilization by HSP90-CDC37 interaction did not work for primed and closed-form LCK. The importance of the effect demonstrated experiments using geldanamycin as a specific ligand for the ATP-binding site of HSP90-CD37, which prevents the LCK binding. It resulted in significant degradation of LCK molecules in T cells activated in the presence of geldanamycin[35]. To establish an optimal level of active LCK in T cells, LCK molecules not bound to the HSP90-CD37 undergo ubiquitinoylation, which directly targets them to the proteasomal degradation[66].

E3 CBL ligase is highly specific and seems to have the most critical rule in the ubiquitin attachment and therefore is essential in the regulation of LCK activity[64]. LCK interacts with CBL via SH2 and SH3 domains, which contact proline-rich regions within CBL[15]. Experiments with kinase-dead LCK (D273A) and a constitutively activated LCK mutant (Y505F) resulted in the conclusion that only the Y505F LCK is likely to be used as a substrate for CBL ligase, while the kinase-dead LCK remains successfully resistant to the ubiquitin attachment[66]. The mechanism of LCK activity regulation focuses explicitly on the molecules in an open conformation with the SH3 domain released. It is a highly specific degradation of activated LCK molecules only, while the inhibited LCK molecules remain unaffected. Thus, CBL ligase

enables to control of the number of active LCK molecules for initiating appropriate signal transduction after TCR stimulation[66].

## 4.5 Regulation of LCK activity within membrane microdomains

LCK can interact with LAT directly, without the intermediate interaction with ZAP-70[67]. Nevertheless, the interaction was observed only within the detergent-resistant microdomains, where measurements of LCK activity shown a dramatically decreased number in comparison with the cytosolic LCK. They logically reasoned the drop in LCK activity as a result of CD45 exclusion from these microdomains, which consequence is, that LCK cannot be released from its closed state. Nevertheless, surprisingly the authors could also detect several open, thus active LCK molecules. The open form of LCK binds LAT more efficiently than closed forms. Taken together, the LCK-LAT interaction within these membrane structures has a function to negatively regulate the active LCK molecules and ensure the prevention of unwanted ITAM phosphorylation within unstimulated TCRs[67].

PAG is an adaptor molecule, and by its activity, LCK is kept in an inhibited state[68]. PAG becomes active when it is phosphorylated on its cytosolic domain. When PAG is activated, it interacts with the SH2 domain of CSK and recruits it to the microdomain. CSK recruitment leads to the desired dephosphorylation of LCK on pY394 and therefore adopting the closed conformation[67]. The recruitment of CSK to the microdomain is made via palmitoylation of the intracellular domain of PAG[15]. Brdička et al. also demonstrated that PAG phosphorylation is highly dependent on the LCK activation state. They used an LCK-negative mutant of Jurkat T cells and observed a significantly reduced PAG phosphorylation. In the other case, where they used ZAP-70-negative Jurkat T cells, no reduction in PAG phosphorylation appeared. So it was concluded that PAG phosphorylation status is under control of the level of LCK activity[68].

PAG is constitutively interacting with CSK in resting T cells[69]. The interaction enhances the PTK activity of CSK and protects the kinase from dissociating away from the membrane raft[70]. However, it is not a suitable environment for signal transduction. For this reason, PAG is temporarily dephosphorylated and releases the CSK immediately after the onset of TCR stimulation. After the CSK-PAG interaction is disrupted, CSK cannot phosphorylate the inhibitory tyrosine within the carboxy-terminus of LCK, because it does not associate with the membrane microdomain anymore[68]. This temporary release of CSK from the detergent-resistant microdomain prevents uncontrolled TCR signaling interruption.

## 4.6 Other ways of regulation

Granum et al. described how a T cell-specific adaptor protein (TSAd) could participate in TCR signaling regulation[18]. The adaptor possesses three tyrosines of phosphorylation within its C-terminus, while all of them can serve as a substrate for LCK. The surprising observation was that the TSAd requires both the SH2 and SH3 domain of LCK to interact with it. The multisite interaction with LCK could be somehow important for LCK activity modulation and for setting up its microenvironment in cell[18]. Another study revealed that TSAd can have a role in promoting of LCK activation during TCR stimulation[71]. However, the exact mechanism of the activation remains unexplained.

# 5 Coreceptor bound LCK enhances TCR signaling

Can LCK alone convert a ligand binding event into tyrosine phosphorylation of the CD3 complex? Some publications have distinguished a TCR phosphorylation provided by LCK to coreceptor-dependent and coreceptor-independent[72],[73]. Both ways of TCR signaling initiation can lead to sufficient signal propagation. However, coreceptor-dependent TCR signaling shown to be more efficient at even lower antigen doses and affinities[73].

Nevertheless, in the case of lower affinity antigens and transient signals[28], LCK requires interaction with either CD8 or CD4 coreceptors depending on the MHC I or II, respectively[74]. Both of these CD4 and CD8 coreceptors possess the same modifications as LCK, particularly palmitoylation, which allows them to incorporate to the membrane sheet and localize the microdomain with the immune synapse[15]. The coreceptor and LCK interact via the dicysteine motif within the N-terminal sequence of LCK with another two cysteines within the intracellular domain of the coreceptor[1]. The structure and function of the N-terminal domain are unique for LCK within the Src kinases[8]. A $Zn^{2+}$ ion provides coordination of the whole complex[75]. Nevertheless, the exact mechanism of how the disruption of zinc clasp coordinated complex allows the LCK dissociation and coreceptor internalization remains unclear.

## 5.1 The way, how coreceptors help

The T cell and APC adhesion and formation of a complex consisting of pMHC, TCR, and a coreceptor have shown to include two phases. First, TCR interacts with pMHC, and subsequently, the TCR-pMHC complex is stabilized by a coreceptor interaction[72]. That is the reason why the coreceptor interaction enhances signaling in two aspects. The first aspect is that the association of pMHC with CD8 (but not with CD4) can increase the stability of the pMHC-TCR complex. The coreceptor bound with pMHC couples with the LCK within the T cell, and the LCK interacts with the CD3 complex of TCR — such mechanisms of multiple interactions of a T cell with an APC help to prevent undesirable diffusion and signaling abortion[13].

Another group has supported the two-stage coreceptor-dependent adhesion of an APC and a T cell after TCR triggering [76]. The TCR must interact with the very same molecule of pMHC as the coreceptor to upregulate the second stage of the cell adhesion, and they referred to this interaction as a cooperative binding. The model of cooperative interaction between an APC and a T cell excludes the previously published model of single-stage competitive adhesion[77], where TCR and coreceptor create bounds with the pMHC independently on each other. Interestingly,

inhibition of LCK led to the significantly lowered frequency of the second stage of cell adhesion. The possible explanation of the effect is that the coreceptor, coupled with non-functioning LCK, could not reach the TCR via ITAMs engagement[76].

The second one and primarily essential effect are that the coreceptors manage the recruitment of the associated LCK to the immune synapse locus[13]. Both coreceptors possess a binding site that can bring LCK to the proximity of the ITAMs by the engagement of the pMHC bound to the triggered TCR[72]. The coreceptor coupled LCK does not diffuse that quickly from the triggered TCR, and therefore it is an essential step in the kinetic proofreading process[78].

CD8 coreceptor possesses an affinity to MHC class I higher than CD4 to MHC class II, about 2 to 4-fold[13]. Consequently, CD4 dissociates more efficiently from the forming synapse and cannot provide the stabilization of the TCR-pMHC complex[13]. On the other hand, CD4 can bind LCK more efficiently than CD8, which makes the CD4 mediated signaling through a TCR stronger[79]. It is consistent with the findings that in CD4+CD8+ thymocytes (DP) is much more LCK molecules bound to CD4 than CD8 coreceptor[80]. The result was supported by an experiment, where the CD8 cytoplasmic tail was replaced with the one originally from the CD4 coreceptor, the intensity of the signaling raised[79].

The mechanism of LCK-coreceptor coupling and its impact on the T cell signaling has always been a subject of discussion. Some publications highlight the role of coreceptor co-clustering with the TCR in the recruitment of active coreceptor-bound LCK to the immune synapse. The stabilization of the pMHC-TCR complex is thus explained as a secondary effect. It is supported by the fact that only the CD8 can provide the stabilization[13].

However, another group published a slightly different results[72]. The authors assumed that LCK involved in the initiation of TCR signaling occurs in two forms within a cell. They described a free LCK and LCK-bound to a coreceptor. Free LCK should provide the initial ITAM phosphorylation. The phosphorylated ITAMs then attract the other LCK molecules to the TCR via its SH2 domains, including the coreceptor bound. These molecules of the coreceptor-associated LCK finally recruit a coreceptor to stabilize the complex of already phosphorylated TCR-pMHC[72].

However, Casas et al. used OVA peptide in the experiment, which is known to be a higher affinity antigen, and thus can potentially initiate the T cell stimulation even in the absence of a coreceptor.

So there are two models of TCR signaling initiation. The first one, that coreceptors directly recruit associated LCK to the synapse versus the other one highlighting that a free LCK provides the necessary step of initial ITAM phosphorylation, which is essential for subsequent coreceptor-associated LCK recruitment and stabilization of the TCR-pMHC complex.

A recent study published data consistent with Casas's results[81]. They used LCK-knock-out hybridoma cells expressing OVA reactive TCR (OT-1) that were reconstituted with either CD8-binding mutant of LCK $C_{20}$/A $C_{23}$A (substitution of cytosines 20 and 23 to alanines) or LCK permanently bound to CD8α. After stimulation of these cells, the coreceptor-bound LCK shown significantly lower mobility within the cell than the coreceptor-not bound. They reasoned the effect as a consequence of the smaller size of free LCK, and the fact, that free LCK binds only with the inner leaflet of the membrane[81].

Moreover, the pool of free LCK possessed a much higher ratio of Y394 phosphorylation than the coreceptor-bound LCK pool, and it did not change even after a TCR stimulation[81]. The free LCK also has shown a higher kinase activity than the coreceptor-bound. However, phosphorylation of the LCK downstream molecules such as ZAP-70 and LAT was comparable in these two pools. These recent findings are in agreement with the Casas's results. In the model, free LCK provides the initial CD3 phosphorylation before the coreceptor-bound[81] and that the coreceptor-bound LCK enhances the initiated signaling or stabilizes TCR: MHC interactions[16].

## 5.2 The effect of coreceptors during thymocyte development

DP thymocytes are also capable of signaling even after the deletion of both coreceptors. However, these possessed decreased self-tolerance in mature stages[73]. The coreceptor-LCK association during the DP thymocyte development is probably more important for providing of MHC-specificity recognition than for general signaling thought TCR[73].

During the development in the thymus, LCK drives a TCR expression and distribution within the plasma membrane in CD4+CD8+ thymocytes. MHC class II-dependent interaction with CD4 coreceptor provides the ability of LCK to regulate such processes[80].

The presence of CD8 surface antigen and the strength of signaling thought it is crucial for establishing of a CD4+: CD8+ T cells ratio during a T cell development[79]. The mechanism, that drives this ratio establishment is an increased positive selection of MHC class I-specific CD8+ T cells. Thus it was refused to be a result of a lineage fate modulation[79]. On the other hand, a coreceptor-independent TCR signaling during positive selection, which does not include a

specific coreceptor-MHC interaction, leads to preferential production of CD4+ T cells over CD8+ T cells[73].

Surprisingly, the selection in the thymus can be partially MHC-independent[82]. Whether the selection will be MHC-restricted or not is probably determined by the occupation of LCK within the cell. LCK associated with a coreceptor preferentially mediates the selection of conventional MHC-restricted αβTCRs while the presence of free-form of LCK during the thymocyte development enhances the selection of MHC-independent γδTCRs[82].

## 5.3 The portion of coreceptor associated with pY394-LCK

Many controversial opinions about the abundance of LCK-coreceptor interaction appeared. One study suggested that approximately 50 % of all CD4 molecules do non-covalently associate with LCK and that the interaction sustains even in a cell lysate of murine and human CD4+/CD8+ T cells (Veillette, Bookman, Horak, & Bolen, 1988),[74].

The other group has published that LCK: coreceptor coupling is different in naive, effector, and memory T cells[84]. The ratio of LCK: coreceptor coupling should differ dependently on the effect of CD28 costimulation signal within these subpopulations of T cells. Only naive T cell responses were enhanced by CD28 costimulation while the effector and memory T cells remained unaffected. They reasoned the drop in the affection of CD28 as the result of increased TCR signaling amplification due to a higher rate of LCK: coreceptor coupling within effector and memory T cells. LCK in naive T cells is homogeneously distributed within the cytoplasm while LCK in effector and memory T cells have a much higher rate of coupling with the CD8 coreceptor, which location allows a rapid reaction to the TCR triggering[84].

Stepanek et al. published, that most of the coreceptors in a T cell do not bind LCK at the moment. The fact makes coreceptor-LCK coupling the most proximal step, that limits and controls a TCR signaling during negative selection in thymocyte development. The study concluded that a TCR has to "scan" up to hundreds of coreceptors until it finally finds one that is active-LCK coupled and can potentially initiate signaling through the TCR. Their preferred model, "LCK come&stay/signal duration," was based on the TCR kinetic proofreading rules of TCR signaling[85], and it supports the hypothesis that LCK-coreceptor remains associated with a TCR until the stimulating antigen is associated with the TCR[86].

Moreover, LCK-coreceptor coupling is probably a highly dynamic process, and the number of coreceptors interacting with LCK changes during the thymocyte development differently in CD4+ or CD8+ T cells[87]. A higher rate of self-reactivity in CD8+ T cells is probably based on

different LCK-coreceptor coupling in DP stages. LCK-CD4 stoichiometry is higher than LCK-CD8 in DP thymocytes because LCK preferentially couples CD4 due to the higher affinity compared to CD8. However, after the maturation, the LCK-CD8 coupling rate significantly increases. Thus, different LCK-coreceptor coupling within CD8+ and CD4+ T cells is crucial for establishing self-reactivity levels in these populations[87].

It suggests an explanation that with decreased LCK-coupling in the DP stage, CD8 coreceptor loses its advantage before CD4. Although, CD8 can bind the MHC I glycoproteins more efficiently than CD4 binds MHC class II, the affinity of CD8 to LCK is lower. Thus, the probability that LCK would associate with CD8 is also lower than with CD4, and even if it does, the formed TCR-pMHC-CD8 complex will possess a decreased stability compared to TCR-pMHC-CD4. Thus, the interaction of self-MHC I and the TCR in DP thymocytes could be disrupted before the antigen recognition is completed and it can lead to the production of self-reactive CD8+ T cells responding to the insufficiently presented self-antigen.

# 6 Existence of an adaptor role of LCK in T cells

The non-enzymatic function of LCK is definitively the least understood. However, a kinase-independent function has shown in CD4 associated LCK molecules[88]. LCK was involved in assembling other signaling complexes, and proteins like Crk and Sem-5/GRB2[88] in these cells.

The studies with CD4-LCK chimeric molecule revealed that the T cell was able to respond to an antigen stimulation even after the removal of the LCK kinase domain[88]. The fact suggests a general conclusion that LCK's role in T cell signaling is not strictly dependent on its catalytic activity, and the non-enzymatic attributes of the molecule can partially contribute to some of the functions and protein-protein interactions[88].

The ability to serve as an adaptor and form such signaling complexes is probably enabled by the structural properties of the SH2 domain of LCK[78].

Moreover, it has shown that LCK bound to a CD4 coreceptor also serves as a scaffold for PI-3K and recruits it to the proximity of triggered TCR[89]. PI-3K interacts with the LCK-CD4 complex via the SH3 domain of LCK. PI-3K activation thus depends on the enzymatic and nonenzymatic function of LCK, respectively. However, the similar function in PI-3K recruitment was observed in Gab2[89].

The conclusion of the model published form Casas that the formation of the TCR-pMHC-CD8 complex has two stages[72] is consistent with the idea, that LCK provides a scaffolding activity. While the complex of pMHC-TCR forms within the first stage, the CD8 is recruited in the second one. LCK seems to be involved in CD8 recruitment. It probably serves as a scaffold protein that attracts the coreceptor to the formed pMHC-TCR. It was supported by the experiment, where the cells expressing $C_{20}$/A $C_{23}$A CD8-binding mutant of LCK could not reach this CD8 recruitment[72].

Many experiments have shown that ZAP-70 and LAT aggregate nearby the stimulated TCR. One possible explanation of the effect could be the existence of a scaffold or an adaptor function of activated LCK, that recruits them[21].

# 7 Conclusions

The thesis answered the aimed questions of how important LCK is in the process of T cell activation and thymocyte development. It has described the structure of LCK, the mechanisms that evoke the change in LCK conformation, and the principles of how the conformational changes modify LCK activity. The importance of the coreceptor-LCK interaction in T cell activation and during T cell development has been definitively emphasized. It provides evidence that LCK also mediates various signaling pathways affecting T cell and NK-mediated cytotoxicity and a conclusion of published data relating to the LCK-coreceptor dynamics in the thymocyte development and its effect on self-antigen responsiveness.

Although LCK has been a subject of research for more than 30 years, several circumstances still appear about it. The controversial opinions relate to the questions of what portion of LCK is constitutively active in unstimulated T cells. It seems that unstimulated T cells possess the preactivated pool of LCK and these constitutively active LCK molecules involve the strength of signaling through the TCR. However, the initial idea that the amount of preactivated LCK is around 40 % of the entire LCK pool is already overcome. More recent publications reveal that the portion of preactivated LCK is significantly lower. Maybe more controversial thoughts appear around the coreceptor-LCK interaction. Currently, it seems, that the rate of LCK molecules coupled to a coreceptor is not even that high as was originally thought. Moreover, the differentiation of the TCR signaling into the coreceptor-dependent and coreceptor-independent has also shown to be quite misleading, because recent studies highlight the model, where even the coreceptor-dependent TCR signaling is initiated by free-LCK, thus in the absence of a coreceptor association. In my opinion, it is still not conclusively described whether the coreceptor recruits LCK to the triggered TCR or the coreceptors are recruited after the TCR ITAM phosphorylation, possibly by an adaptor function of LCK.

Especially the adaptor role of LCK and the dynamics of LCK-coreceptor interactions need more investigation to be better explored. Such understanding could expand the recent knowledge about LCK behavior within a T cell and could potentially be used in the treatment of various health conditions, including autoimmunity disorders.

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