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T-lymphocyte signalling in systemic lupus erythematosus: a lipid raft perspective

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Abstract

In the last few years it has become clear that in cells of the immune system, specialized microdomains present in the plasma membrane, called lipid rafts, have been found to play a central role in regulating signalling by immune receptors. Recent studies have looked at whether lipid rafts may be connected to the abnormalities in signalling seen in T lymphocytes isolated from patients with systemic lupus erythematosus (SLE). These early findings show that in SLE T cells, the expression and protein composition of lipid rafts is different when compared with normal T cells. These results also demonstrate changes in the function and localization of critical signalling molecules such as the LCK tyrosine kinase and the CD45 tyrosine phosphatase.

Keywords

CD45; intracellular signalling; LCK; lipid rafts; T lymphocytes

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease with a highly variable and unpredictable progression. Patients can present clinically in many ways, with abnormalities observed in musculo-skeletal, cardiopulmonary, gastrointestinal, renal, cerebral, haematological and dermatological systems.1 The principle underlying disorder is an impaired humoral and cellular immune response to self-antigens resulting in the characteristic abnormal production of autoantibodies to nuclear components. However, despite extensive research, the precise actiopathogenesis of SLE remains unclear. Virtually every aspect of the immune response to antigen has been shown to be abnormal in patients with SLE and current evidence suggests a central role is played by underlying defects in T lymphocyte antigen receptor mediated signalling pathways leading to the loss of tolerance to self-antigens.2,3

Abnormalities in immune cell function in SLE have been confirmed by the pattern of gene expression produced from oligonucleotide microarray analysis of peripheral blood mononuclear cells (PBMCs) from SLE patients. In one study, approximately 50% of adult patients with SLE had upregulated interferon (INF)-α associated genes4 and an important study of paediatric patients with severe lupus revealed recently that all patients demonstrated INF-α regulated gene expression. Fourteen out of the 15 genes over-expressed in PBMCs from these patients were identified as targets of INF-α activation including genes for upregulation of auto-antigens Ro and lamin, complement components C2 and C1 inhibitor

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and molecules associated with dendritic cell (DC) maturation and antigen presentation.5 Genes associated with granulopoiesis were also upregulated and expression of INF-α regulated and neutrophil encoded genes correlated with disease activity.5

Many mechanisms influencing T cell tolerance have been implicated in the actiopathogenesis, of SLE, these include abnormalities in DC maturation, deletion of self-reactive cells, and defects in regulatory T cells and T cell intracellular signalling pathways. Several excellent reviews have discussed these mechanisms in detail.6 11 Nonetheless, work performed recently, investigating lipid raft function in SLE T cells, has provided further insight in to the signalling abnormalities that may contribute to loss of peripheral T cell tolerance in patients with SLE.12 14 This review will focus, therefore, on the role of lipid rafts and associated signalling molecules and how these domains may determine the threshold for T cell activation in this disease.

Proximal T cell signalling events

The immediate consequence of T cell receptor (TCR) ligation by specific MHC/peptide is a rapid increase in protein tyrosine phosphorylation mediated by the sequential action of LCK and ZAP-70 tyrosine kinases, which are responsible for the initiation and amplification of the activation signal. In quiescent T cells, phosphorylation of ITAMs (immunoreceptor tyrosine based activation motif) present on the TCR/CD3 chains, by a pool of active LCK is an ongoing reaction balanced by the presence of protein tyrosine phosphatases, for example CD45.15 TCR activation may shift the balance of phosphorylation by excluding phosphatase molecules from the vicinity of the TCR by formation of the immunological synapse therefore promoting ITAM phosphorylation.16

The main substrate for active ZAP-70 is the transmembrane adaptor molecule LAT (linker for activation of T cells), which becomes phosphorylated at multiple positions.15,17 Phosphorylated LAT nucleates protein complexes, the formation of which is critical for propagation of the TCR signal. LCK and LAT are constitutively partitioned within specialized membrane microdomains, termed lipid rafts, and upon TCR ligation, many proteins are targeted to lipid raft domains by protein–protein interactions triggering downstream signalling pathways.18 These include activation of the Ras–MAPK (mitogen activated protein kinases) pathway and hydrolysis of phosphatidylinositol 4,5-biphosphate by phospholipase Cγ1 (PLCγl) leading to intracellular calcium (Ca^{2+}) release. Activation of these and other pathways results in gene transcription, cytokine synthesis and release, activation or apoptosis19,20 (Figure 1).

Role of lipid rafts in TCR cell signalling

In the last few years, it has become evident that the organization of signalling molecules into discrete membrane associated microdomains, termed lipid rafts, is vital for the regulation of T lymphocyte activation pathways.20,22,23 Lipid raft domains consist of assemblies of tightly packed sphingolipid and cholesterol moieties and were originally defined by their insolubility in cold nonionic detergents (specifically 1% Triton-X-100), which facilitates their isolation by floating in the upper half of a 5–30% sucrose density gradient. Recently, confocal microscopy and other imaging techniques have been used to visualize lipid rafts in intact cells and to provide information about their role during receptor signalling.24 Many of these techniques use cholera toxin B (CTB) subunit, which binds specifically to raft associated glycosphingolipid GM1. This marker has been used widely to detect lipid rafts25,26 and accumulated experimental evidence supports the use of GM1 as a credible indicator for lipid raft domains.

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In T cells, signalling molecules that partition to lipid raft domains include the src-family kinases, LCK and FYN, GTP-binding proteins, GPI-linked receptors, CD4 and CD824 and adaptor molecules LAT and Cbp/PAG (CSK binding protein/protein associated with GEMs).18,27,28 Transient incorporation of other receptors and signalling proteins has also been observed during TCR activation, including the TCR itself.24,29,30 Some reports also suggest that CD45 may copurify with lipid rafts although other reports found CD45 to be excluded from these domains.31,32

In naive and resting T cells, the proportion of the plasma membrane adopting a raft conformation is low, measured by expression of the raft associated sphingolipid, GM1. TCR activation induces GM1 synthesis, and targets cytosolic LCK to the plasma membrane, thereby increasing the availability of lipid rafts and associated signalling molecules.26 Although their role in the formation of the immunological synapse is not fully understood, current evidence places lipid raft domains and associated signalling molecules at the centre of the supramolecular activation complex (cSMAC).33 Costimulation by CD28 bolsters signalling via the TCR by upregulating lipid raft expression and initiating changes in the actin cytoskeleton that stabilizes the T cell/antigen presenting cell (APC) contact area.34 It is interesting to note that lipid rafts are enriched with the cytoskeletal component, F-actin,35 thus changes in the cytoskeleton, stimulated by CD28 engagement, are likely to control the polarization of lipid rafts and their associated signalling molecules to the immunological synapse.36 Conversely, signalling via the inhibitory coreceptor CTLA-4 blocks surface upregulation of rafts.37 Thus the mechanism via which CD28 and CTLA-4 control T cell activation may, at least in part, be due to their ability to regulate lipid raft expression and their association with the immunological synapse.24,37

T-lymphocyte activation needs to be strictly regulated to maintain the specificity and fidelity of the immune response. The expression of lipid rafts is central to the control of T cell signalling pathways suggesting that alteration in the properties or composition of lipid rafts may lead to inappropriate T lymphocyte signalling and result in the development of pathological conditions including autoimmunity.

Abnormal intracellular signalling in T cells from patients with SLE

The threshold for T cell activation is lowered in patients with SLE.38 Moreover, defects in the expression and function of a number of signalling molecules have been reported, which may be responsible for the heightened sensitivity and the prolonged response to TCR activation seen in T lymphocytes from lupus patients.3 Principally, these defects involve molecules associated with early TCR/CD3 complex and CD28 costimulation pathways, although abnormalities have also been described in downstream signalling molecules and nuclear transcription factors.3,11 A summary of the T cell intracellular signalling abnormalities described in patients with SLE is shown in Table 1.

Altered expression of the lipid raft associated glycosphingolipid GM1

T cells freshly isolated from patients with SLE were found to express higher levels of the glycosphingolipid GM1, a constituent of lipid rafts.13 Increased levels of GM1 most likely indicate that a higher proportion of the plasma membrane assumes a lipid raft structure and could be linked to the phenotype manifested by SLE T cells. An increase in lipid raft formation could increase the strength of the T cell signal by reducing the threshold for T cell stimulation. This in turn may influence immune cell function in SLE patients, and, as such, lipid rafts may provide targets for future therapy.24 Lipid raft heterogeneity, in terms of both protein and lipid content, has been described in relation to T cell activation and function.26,39-42 Thus, decreased lateral mobility of T cell surface receptors and reduced

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capping in T cells from patients with SLE may reflect abnormalities in lipid raft composition.43 Increased lipid raft expression has also been reported previously in activated and memory T cells compared with naive T cells.25,26 Thus the increased raft expression seen in SLE T cells could be indicative of an 'activated' phenotype which may result from exposure of these cells to differentiated dendritic cells expressing relevant coreceptors, cytokines, circulating immune complexes or a combination of these factors.

Expression and regulation of LCK in SLE T cells

Aberrant expression and tyrosine phosphorylation of LCK have been described previously in PBMCs from patients with SLE and it has been suggested that LCK and FYN have increased activity in these cells.44-47 These results were confirmed recently by another report showing that expression of LCK was significantly reduced in both raft and non raft fractions in T lymphocytes from patients with active disease compared with patients with rheumatoid arthritis (RA) and healthy controls.12

LCK is essential for T cell differentiation and signalling48 and its partition to lipid rafts via a unique domain at the N terminus, is essential for its function.49 Experiments using LCK deficient cell lines indicate that LCK is responsible for most, if not all, of the ITAM phosphorylation that follows TCR engagement.50 Tsokos and colleagues have described reduced expression and mutations of the TCR-ζ chain in lupus T cells which may affect their activation,10,11 although the chains of the CD3 complex, which also contain ITAMs, may compensate for the reduced TCR-ζ expression.51 Interestingly, SLE T cells express the Fc receptor gamma chain (FcR-γ) which may make up for the absence of TCR-ζ.52 In addition to its kinase activity, the SH3 and SH2 domains of LCK interact with a wide range of adaptor and signalling molecules including the ubiquitin ligase c-Cbl and costimulatory molecules CD28 and CD2 that mediate remodelling of the cytoskeleton and assembly of raft domains within the immunological synapse.53,54 Thus, reduced expression of LCK in SLE T cells is likely to affect immune function. Little is known about the effect of reduced LCK on the function of primary T-lymphocytes. In LCK deficient mice, LCK is critical for the generation and maturation of thymocytes since these animals develop very few T cells, which are phenotypically abnormal.48 Absence of LCK in peripheral blood T cells leads to defects in their proliferative potential55 and diminished numbers of $CD8^+$ cells, poor responses to CD28 costimulation and reduced calcium mobilization after stimulation.56 The effect of reduced LCK in patients with SLE is not clear, however, a similar reduction has been observed in patients with type I diabetes57 and conformational changes affecting LCK function have been reported in synovial T cells from patients with RA.58 In both cases, abnormal LCK expression was associated with T cell hyporesponsiveness. Correspondingly, lupus patients also show considerable evidence of reduced T cell proliferative responses to recall antigens.59

Reduced LCK expression in lupus T cells was found to be independent of the treatment regime or the activation status of the cells as determined by expression of the activation markers CD69, CD25 and CD95.12 However, there was increased ubiquitination of LCK in SLE T cells compared with normal controls, which could result in accelerated proteasomal degradation of the molecule. This observation supports recent studies showing an increased ubiquitination of CD3-ζ chain in T cell lysates from lupus patients.14 Protein ubiquitination targets proteins for degradation by the 26S proteasome and is a critical process regulating signal transduction in eukaryotic cells,60 thus the increased level of ubiquitinated proteins and ubiquitinated LCK may be the result of continuous T cell activation by autoantigens in SLE. However, this seems unlikely as LCK was reduced in both activated and nonactivated lupus T cells. In addition, SLE T cells activated *in vitro* with antibodies to CD3 and CD28 did not show differences in LCK expression over a 60 minute time course compared to

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controls, although differences in protein ubiquitination and phosphorylation were observed. These findings suggest that T cell activation is not solely responsible for the low levels of LCK observed in T cells from patients with SLE.12

Abnormal regulation of LCK activity in T cells from patients with SLE

Abnormal LCK ubiquitination and phosphotyrosine profiles observed in lipid rafts might indicate that the pool of LCK is dysregulated in patients with SLE.12 The precise regulation of LCK is critical for maintaining T cell homeostasis and for initiating activation of signalling cascades. There is good evidence that LCK function is controlled by the reciprocal action of protein tyrosine phosphatase (PTPase) CD45 and the protein tyrosine kinase c-terminal src kinase (CSK)61,62 (Figure 2). Cbl, an E3 ubiquitin ligase, also plays an important regulatory role in the immune response and is associated with negative regulation of active LCK.63,64 Precise LCK regulation ensures that the immune system can respond rapidly to foreign antigen. However, an imbalance in its regulation may predispose T cells to autoimmunity.65

LCK conformation and kinase activity are regulated by the phosphorylation of two important tyrosines, an autophosphorylation site (Tyr-394) and an opposing negative regulatory site (Tyr-505). Phosphorylation of the negative regulatory site in the carboxy tail induces an intramolecular interaction with the SH2 domain, whereby the molecule folds to adopt an inactive or closed LCK conformation. This so-called ‘tail-bite’ structure is further stabilized by interaction of the SH3 domain with the linker region. Dephosphorylation of Tyr-505 generates an open conformation, where the SH2, SH3 and kinase domains are exposed and available for intermolecular interactions. Autophosphorylation of Tyr-394 in the kinase activation loop, displaces the loop from the kinase active site, resulting in greater accessibility to the site and full kinase activation66 (Figure 2).

The role of CD45

The CD45 phosphatase plays critical and diverse roles during T cell signalling. It positively regulates src-family PTKs, such as LCK, by dephosphorylating the negative regulatory residue Tyr-505, resulting in the kinase adopting an active conformation. In the resting T cell. CD45 maintains LCK in a primed state ready to respond to TCR activation. But CD45 can negatively regulate TCR signalling by dephosphorylating the TCR-ζ chain.67 It is also possible for CD45 to dephosphorylate Tyr-394, thus reducing LCK activity, although the latter is not believed to be the dominant interaction.68

The possibility that reduced LCK expression results from dysregulated LCK homeostasis in T cells from SLE patients has been reported recently.13 An increase in the localization of CD45 to lipid raft domains is observed along with a parallel increase of activated LCK in these domains. Also, an increase in the physical association of CD45 with LCK has been detected in SLE T cells compared with controls.13 The location of CD45 is important for its function as a regulator of LCK activity. A body of evidence suggests that CD45 is excluded from lipid rafts and that it regulates LCK activity by its proximity to raft domains. 32,61,68,69 However, recent reports have shown that CD45 is associated with T cell lipid raft domains in T cell lines and that it is excluded only after T cell activation,70,71 thus suggesting that the lipid raft association of CD45 is dependent upon the activation status of the cell. T cells from patients with SLE demonstrate a more pronounced inclusion of CD45 to raft domains, and CD45 is maintained in these domains even after stimulation of the TCR, pointing to the possibility that alterations in the distribution of CD45 in lupus T cells may contribute to the abnormalities seen in these cells.

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The consequences of increased LCK activation in lipid rafts, if LCK levels remain above a critical threshold for signalling, may result in hyper-responsive T cells, primed to respond to TCR/CD28 stimulation more readily. This assumption is confirmed by the correlation between increased levels of active LCK, and increased protein tyrosine phosphorylation following T cell activation13 and increased calcium mobilization in SLE.72 T cells from patients with SLE are therefore 'primed' for activation and respond more rapidly to antigenic triggers than T cells from normal controls. This may explain the exaggerated response to environmental antigens observed in SLE patents.73 However, other reports show reduced CD45 activity in SLE,46,75 which may be associated with an altered CD45 phenotype.59 The activity of CD45 is regulated by dimerization.75 The CD45RO isoform (associated with memory or activated T cells), dimerizes more readily than CD45RA (associated with naive/resting T cells). The phosphatase domain of CD45RO forms a symmetrical dimer in which the catalytic site of one molecule is blocked by specific contacts with a wedge from the other (reviewed in Hermiston *et al.*68). Furthermore, *in vivo* experiments using 'knock-in' mice with an inactivating point mutation in CD45 prevent the formation of the inhibitory wedge and develop lymphoproliferation and lupus-like nephritis. 76 It will be interesting to investigate whether CD45 dimerization is different in lupus T cells compared with controls.

Evidence from fluorescent resonance energy transfer (FRET) analysis has shown that CD45RO localizes preferentially with raft partitioned CD4 and CD8 and is associated with an increase in protein tyrosine phosphorylation.77 Furthermore, CD26, a raft associated molecule with increased expression in activated and memory T cells, binds the cytoplasmic domain of CD45RO, bringing it to close proximity with LCK and increasing LCK activity. 78 The distribution of CD26 and CD45RO can be affected by cytokine signalling. For example, the ThI type cytokine IL-12 affects the distribution of CD45RO by excluding it from lipid rafts.79 These observations support the idea that low molecular weight CD45 isoforms associate with rafts, but larger molecular weight isoforms are excluded thereby influencing CD45 PTPase activity. Thus, the activation of LCK by CD45 depends upon the membrane location and isoform of CD45, which, in turn, is influenced by T cell maturation and the cytokine environment.

The role of CSK

The action of CD45 on LCK phosphorylation is balanced by CSK, a 50 kDa soluble PTK that interacts via its SH2 domain with the raft bound adapter protein, Cbp/PAG, and downregulates LCK activity in lipid rafts.28,61 The most prominent tyrosine phosphoproteins in lipid raft domains from unstimulated T cells are LCK and Cbp/PAG. Whether CD45 can dephosphorylate Cbp/PAG, in addition to LCK, leading to dissociation of CSK in lipid raft domains, is currently unknown. Although no alterations in the association of CSK with lipid raft domains have been observed,13 the possibility that association of CD45 with lipid rafts in SLE T cells increases LCK activity not only by dephosphorylating Tyr-505 on LCK, but also by dephosphorylating PAG/Cbp and thus disrupting the CSK-PAG/Cbp inhibitory complex, cannot be excluded.

In 1994, Kammer and colleagues reported deficiency of the PKA isoenzyme in SLE T cells. 80 PKA, a cAMP dependent protein kinase, is an important membrane associated immune cell modulator that increases the activity of CSK, when the latter is bound to Cbp/PAG in lipid raft domains.81 Downmodulation of PKA activity diminishes the negative regulatory feedback mechanism of T cell activation.82 CSK activity is regulated by raft associated PKA and by its interaction via its SH3 domain to cytoplasmic PTPase molecules.61 PKA induces a 2–4-fold increase in CSK activity through phosphorylation of serine(Ser)-364 in CSK.83 Thus, abnormalities in PKA function could contribute to abnormal LCK homeostasis by reducing CSK activity in T cells from patients with SLE.

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Cbl family proteins and T cell activation

The c-Cbl and Cbl-b members of the Cbl-family (Casitas B cell lymphoma-b) of proteins have been identified as E3 ubiquitin ligases and shown to be important in setting signalling thresholds in peripheral T cell responses. Both c-Cbl and Cbl-b contain highly conserved N-terminal phosphotyrosine binding domains (SH2) and a zinc binding domain (RING finger motif) which recruits E2 ubiquitin conjugating enzymes. In addition, a C terminal proline rich motif can associate with SH3 domains on target signalling proteins. Thus, a dual mode of action has been proposed for the Cbl proteins, one that involves ubiquitination mediated degradation of target proteins, and a second where the function of signalling proteins is directly inhibited following their association to Cbl.84

Specifically it has been demonstrated that c-Cbl promotes TCR-ζ chain and Fyn ubiquitination and degradation.85 T cells from mice lacking c-Cbl consistently demonstrated increased levels of TCR, CD3, CD4 and CD69 expression and hyperphosphorylation of proximal signalling molecules, ZAP-70, LAT and SLP-76.86,87 c-Cbl is also important in the regulation of LCK activity. Hawash and colleagues64 demonstrated that the SH3 domain of LCK binds to non raft associated c-Cbl and its over expression resulted in depletion of LCK from lipid raft domains. c-Cbl has also been shown to directly regulate activated LCK by enhancing its ubiquitin mediated proteasomal degradation via binding to the SH3 domain of activated LCK.63

Cbl-b is also involved in the regulation of T cell activation by promoting the ubiquitination of the p85 subunit of PI3 kinase through binding to its SH3 domain. This modulates its recruitment to CD28 and the TCR complex which, in turn, regulates activation of guanine nucleotide exchange factor protein, Vav-1.85 Studies comparing Cbl-b$^{-/-}$ and with c-Cbl$^{-/-}$ knockout mice (KOs) revealed differences in their T cell phenotype. Cbl-b$^{-/-}$ KOs demonstrate T and B cell hyperproliferation, develop spontaneous autoimmunity and hyperphosphorylation of Vav following TCR stimulation, suggesting that TCR signalling has been cut off from the requirement for CD28 costimulation.84,88 In support of this model, Cbl-b has been shown to act as a negative regulator of lipid raft aggregation and TCR activation in the absence of CD28 engagement.89

In SLE T cells, the increase in LCK ubiquitination and subsequent degradation12 could result from increased consumption of the activated form of LCK, possibly due to the altered localization of CD45 and its increased association with LCK. Interestingly, the expression of c-Cbl is reduced in the lupus T cells.13 This observation is supported by recently described abnormalities in the c-Cbl-mediated regulation of signalling pathways in T cell lines derived from patients with SLE.90 Thus, the reduction observed in c-Cbl molecules may have profound consequences on T cell activation threshold in lupus T cells.

The effect of TCR engagement on SLE T cells

When purified SLE T cells were ‘rested’ *in vitro* the changes seen in LCK, CD45 and lipid raft expression in freshly purified cells were reversed. Addition of SLE serum during the *in vitro* incubation did not prevent this reversion of cell phenotype, suggesting that factors present in SLE serum are not sufficient for reproducing the disease phenotype *in vitro*.13 It is possible that while in the body of the patient, T cells come into contact with other cell types and this interaction is responsible, at least in part, for the observed alterations in the proximal signalling pathways. Relevant to this hypothesis is a recent report by Blanco and colleagues,91 which demonstrated that, CD14^{+} monocytes differentiate into plasmacytoid dendritic cells, under the influence of SLE serum. These results revealed that INF-α, whose levels are increased in patients,92 is a factor driving dendritic cell differentiation and activation in SLE. An increase in the differentiation of plasmacytoid dendritic cells resulting

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in increased presentation of autoantigens to T and B lymphocytes could lower the threshold for T and B cell activation. Hence, when lupus T cells were rested *in vitro*, the proportion of plasma membrane adopting a lipid raft structure, measured by GM1 expression, was reduced. However, lipid raft formation on the cell surface was rapidly expanded following subsequent activation with anti-CD3 and anti-CD28.13

These results corroborate studies by Tsokos and colleagues showing that T cells from patients with SLE are 'rewired' and signal using alternative pathways,10 including signalling via the Fc receptor (FcR)γ chain which, although not present in normal T cells, it is expressed in lupus T cells.52 Therefore, anomalous expression and changes in membrane raft location of signalling molecules could contribute to changes in the threshold for T cell activation, characteristic of individuals with SLE.

Summary

Numerous abnormalities in T cell function have been described in SLE patients, although no collective abnormality is present in all patients. A common feature appears to be a reduced threshold for T cell activation. Thus, defects in intracellular biochemical pathways or the provision of costimulatory signals may be responsible for the heightened sensitivity and the prolonged response to TCR activation seen in T lymphocytes from patients with SLE. Recent work has indicated that abnormalities in the localization of signalling or costimulatory molecules to lipid raft domains could contribute to T cell dysfunction in these patients. It is likely that *in vivo* factors, either soluble mediators or cell–cell interactions or both, influence the abnormal distribution of signalling molecules to lipid raft domains, although the possibility that inherent abnormalities also exist in lupus T cells cannot been excluded. Recent work highlights the importance of lipid rafts in maintaining T cell homeostasis and suggests that altering their integrity could promote untimely protein–protein interactions, which can influence T cell function and possibly break T cell tolerance. Future work exploring the precise reasons underlying the increased expression and altered occupancy of lipid raft domains in T cells from patients with SLE may reveal further insights into the pathogenesis of SLE and uncover possibilities for new therapies.

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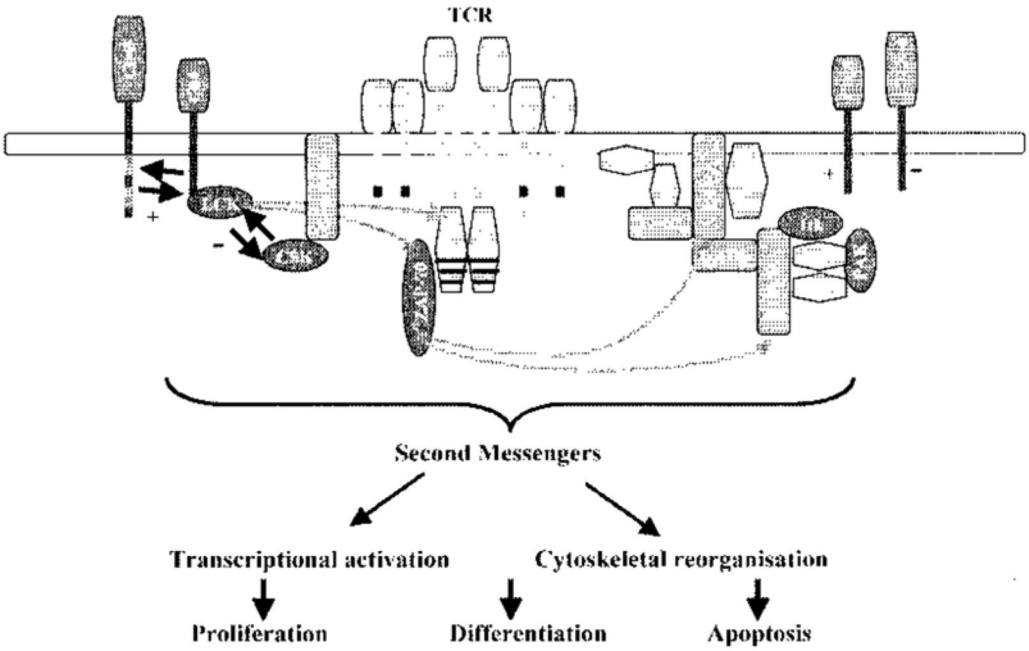

Figure 1.
Signalling is initiated by engagement of the TCR and costimulatory molecules, such as CD28. Phosphorylation of proximal signalling molecules TCR-ζ chain, ZAP-70 and adaptor molecules LAT and SLP-76 by protein tyrosine kinases coordinate the activation of second messenger cascades leading to cytoskeletal reorganization and transcriptional activation. The threshold for TCR activation is regulated by the type of costimulatory molecule expressed, CTLA-4 will downregulate TCR activation, and the regulation of the proximal src-family kinase, LCK. LCK homeostasis is controlled by the reciprocal action of CD45 and CSK. Protein tyrosine kinases are shown in blue, adaptor proteins are orange. Modified from Singer and Koretzky.21

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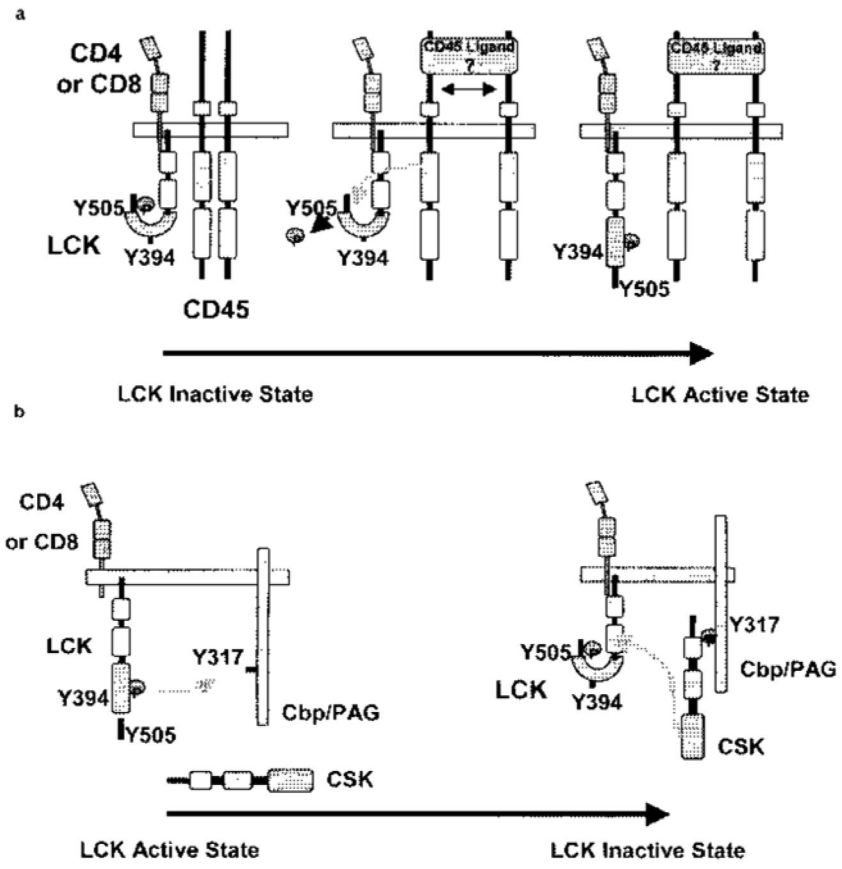

Figure 2.

Regulation of LCK by CD45 and CSK. LCK is controlled by the reciprocal action of the protein tyrosine phosphatase CD45 and the protein tyrosine kinase CSK. a) CD45 dephosphorylates the regulatory Tyr-505 residue, promoting LCK to adopt its open conformation. Subsequently, LCK becomes fully activated by autophosphorylation of Tyr-394. b) Src-family kinases, LCK and FYN, entering lipid rafts phosphorylate the adapter molecule Cbp/PAG resulting in the recruitment of CSK from the cytosol. Recruited CSK phosphorylates raft localized LCK at Tyr-505 inducing its inactive. ‘tail-bite’ conformation.

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Table 1

Summary of T cell signalling abnormalities in patients with SLE

Defects of signal transduction	*Reference*
• Reduced expression of lipid raft associated LCK and increased LCK ubiquitination	12
• Altered association of signalling molecules with lipid raft domains and increased expression of lipid raft associated sphingolipid GM1	13
• Decreased CD3/T cell receptor ζ chain	10
• Increased intracellular free calcium levels	72
• Enhanced tyrosine phosphorylation of proximal signalling molecules	44,46
• Enhanced activity of PTK LCK	45,47
• Decreased CD45 phosphatase activity	74
• Decreased protein kinase A isoenzyme activity	80
• Decreased expression of protein kinase C	11
• Altered mitochondrial hyperpolarization, induction of reactive oxygen intermediates and T cell apoptosis	11
• Increased binding of transcriptional inhibitor pCREM to the IL-2 promoter	10
• Decreased levels of p65-RelA subunit of the NF-κB nuclear transcription factor	10