Immunology 2004 **113** 413–426 doi:10.1111/j.1365-2567.2004.01998.x

REVIEW ARTICLE

Regulation of T-cell receptor signalling by membrane microdomains

TAHIR M. RAZZAQ,* PATRICIA OZEGBE,* ELIZABETH C. JURY,† PHUPINDER SEMBI,* NATHAN M. BLACKWELL* & PANAGIOTIS S. KABOURIDIS* **Bone & Joint Research Unit, William Harvey Research Institute, Queen Mary's School of Medicine & Dentistry, Queen Mary's College, London and †Centre for Rheumatology, Royal Free and University College Medical School, University College, London*

SUMMARY

There is now considerable evidence suggesting that the plasma membrane of mammalian cells is compartmentalized by functional lipid raft microdomains. These structures are assemblies of specialized lipids and proteins and have been implicated in diverse biological functions. Analysis of their protein content using proteomics and other methods revealed enrichment of signalling proteins, suggesting a role for these domains in intracellular signalling. In T lymphocytes, structure/function experiments and complementary pharmacological studies have shown that raft microdomains control the localization and function of proteins which are components of signalling pathways regulated by the T-cell antigen receptor (TCR). Based on these studies, a model for TCR phosphorylation in lipid rafts is presented. However, despite substantial progress in the field, critical questions remain. For example, it is unclear if membrane rafts represent a homogeneous population and if their structure is modified upon TCR stimulation. In the future, proteomics and the parallel development of complementary analytical methods will undoubtedly contribute in further delineating the role of lipid rafts in signal transduction mechanisms.

Keywords microdomains; lipid rafts; signal transduction; T-cell receptor; Lck; adapter

THE T-CELL ANTIGEN RECEPTOR

T lymphocytes recognize antigenic determinants through their T-cell receptor (TCR), a multicomponent structure expressed on their cell surface. The TCR is composed of a highly polymorphic heterodimer (α/β or γ/δ) which detects antigen presented on the surface of antigen-presenting cells (APCs) in the context of appropriate major histocompatibility complex (MHC) proteins.[1,2] The α/β (or γ/δ) chains have a very small cytoplasmic tail and are unable to communicate signals generated by antigen binding. Instead, they are non-covalently associated with the non-polymorphic transmembrane proteins CD3γ, CD3δ and CD3ε (CD refers to cluster of differentiation) and a zeta homodimer (TCRζ). The stoichiometry of proteins in the complete TCR complex is an α/β (or γ/δ) dimer associated with two CD3ε, one of each of CD3γ and CD3δ, and a TCRζ homodimer.[3–5] The CD3 and TCRζ components of the receptor are responsible for transmitting the signal into the cell interior via a structurally conserved amino acid motif present in their cytoplasmic domains. This motif contains paired tyrosine residues and is known as immunoreceptor tyrosine-based activation motif (ITAM).[6–8] Other immune receptors, such as the B-cell receptor (BCR) and the Fcγ immunoglobulin receptor, also use ITAMs to signal.[9] The consensus amino acid sequence of this motif is YXX(L/I)X_{6-8}YXX(L/I) (where Y is tyrosine, L is leucine, and X any amino acid). The TCRζ chain contains three ITAMs in tandem while each of the CD3 chains have one, resulting in 10 ITAMs per single receptor complex. Most likely, the large number of ITAMs present in the TCR has a quantitative role in signal amplification rather than a qualitative role whereby different signals originate from different ITAMs.[10,11] Signalling by the TCR is also facilitated by the CD4 and CD8 coreceptors, which interact with MHC molecules expressed on APCs during antigen presentation (12 and references within).

Productive stimulation of the TCR leads to the activation of a number of signalling pathways that involves

Received 22 June 2004; revised 9 September 2004; accepted 15 September 2004.

Correspondence: Dr P. S. Kabouridis, Bone & Joint Research Unit, William Harvey Research Institute, Queen Mary's School of Medicine & Dentistry, Charterhouse Square, London EC1M 6BQ, UK. E-mail: p.s.kabouridis@qmul.ac.uk

generation of second messengers, increased transcriptional activity and production of new proteins that mediate effector functions of activated T cells.[13,14] Inherent or environmentally imposed changes in the activity of signalling pathways in T cells can lead to pathological conditions such as autoimmunity or alternatively immunodeficiency. Therefore, it is important that signalling homeostasis is maintained precisely. At the plasma membrane, as part of such regulation, is the compartmentalization of signalling proteins and receptors into distinct domains. It is now well documented that initiation and propagation of the TCR-generated signal is critically dependent on the transient assembly and spatial reorganization of such proteins.

TYROSINE KINASES PROXIMAL TO THE TCR

Upon receptor stimulation, the first detectable biochemical event is phosphorylation of the tyrosine residues present within ITAMs. The Src-family kinases Lck and Fyn have been implicated in this phosphorylation.[14–16] Genetic evidence has suggested a more critical role for Lck, as was demonstrated in Lck-deficient cell lines where TCR stimulation failed to trigger ITAM phosphorylation and downstream signalling[17,18] while in $Lck^{-/-}$ mutant mice, T-cell development was blocked at an early stage ($CD4^-$ $CD8^-$) during thymocyte development.[19] Expression of an inducible Lck transgene in the $Lck^{-/-}$ background revealed that when expression of the transgene was switched off in the peripheral T-cell pool the long-term survival of naïve T cells was not affected but their homeostatic proliferation was compromised.[20] Lck interacts with the cytoplasmic tail of the CD4 and CD8 coreceptors. These receptors bind to MHC molecules on APCs during antigen presentation, bringing Lck in proximity to the TCR and thus facilitating ITAM phosphorylation.[21–23]

In contrast to Lck, Fyn seems to plays a more specialized role during TCR signalling since *fyn*-null mice exhibit a defect that is restricted to certain stages of T-cell development.[24,25] Fyn may regulate aspects of T-cell activation by phosphorylating key adapter molecules such as ADAP (adhesion and degranulation promoting adapter protein, also known as FYB (Fyn T-binding protein) or SLAP (SLP-76-associated protein), and SKAP55 (Src kinase-associated phosphoprotein of 55 000 MW).[26–28] These molecular scaffolds form multiprotein complexes during T-cell activation that regulate integrin clustering and adhesion.[29–31] Interestingly, upon TCR stimulation, SKAP55 translocates to lipid rafts where it interacts with Fyn implicating these membrane domains in integrin-mediated adhesion.[32]

Phosphorylated ITAMs form docking sites for the tandem Src homology (SH) 2 domains of the cytosolic tyrosine kinase ZAP-70.[33] Upon recruitment to the TCR, ZAP-70 is phosphorylated, most likely by Lck, and activated.[16,34–36] In humans, absence of ZAP-70 protein, as seen in certain patients, leads to severe immunodeficiency characterized by lack of $CD8^+$ cells while mature $CD4^+$ cells are unresponsive to TCR stimulation.[37–39] In mice, ZAP-70-null mutants reveal a role for this kinase in both positive and negative selection of thymocytes.[40] A substrate for ZAP-70 activity is the adapter molecule LAT (linker for activation of T cells).[41] It contains multiple tyrosine residues in its cytoplasmic domain and when phosphorylated it nucleates multiprotein signalling complexes at the plasma membrane.[41–44] Studies with LAT-negative cell lines showed that while initial tyrosine phosphorylation, including phosphorylation of ITAMs, remained intact downstream signalling events were blocked.[45] Also, thymocyte development was blocked within the double negative stage ($CD4^-$ $CD8^-$) in *lat*-null mutant mice.[46] Proteins that directly bind phospho-LAT include phospholipase Cγ1 (PLCγ1), and the adapter molecules Grb2 and Gads.[41,47] The Tec-family tyrosine kinases Itk/Emt and Txk/Rlk are also involved in PLCγ1 regulation following TCR stimulation.[48,49] Another adapter molecule that participates in these 'signalosomes' through its interaction with Gads is SLP-76, which is also critical in linking early to distal TCR signalling events.[50] Formation of such signalling complexes directs downstream events such as mobilization of intracellular Ca^{2+} and stimulation of the Ras/MAP (mitogen-activated protein) kinase pathway.[51,52] The co-ordinated action of enzymes, adapter molecules and second messengers leads to increased activity of transcription factors like nuclear factor (NF)-AT, AP-1 and NF-κB and expression of new proteins such as CD69, CD25 and interleukin-2 (IL-2).[13] A schematic representation of major participants during TCR signalling is illustrated in Fig. 1.

REGULATION OF LCK

The Src-family of kinases have a conserved structure characterized by distinct functional domains (reviewed in,[53,54]). It includes an amino terminal (N-terminal) motif containing signals for attachment of lipid moieties,[55–57] which in the case of Lck are a myristic acid added cotranslationally to glycine at position 2, and palmytic acid attached to juxtapositioned cysteine residues 3 and 5.[58–65] This dual acylation is sufficient for membrane localization of the protein.[66,67] Following the membrane-targeting motif is a region unique to individual members, which may have specific functions, although such functions remain unclear at present. In the case of Lck, serine and threonine phosphorylation sites have been identified in the unique domain. In particular phosphorylation of serine 59, catalysed by the extracellular regulated kinase (ERK) MAP kinase (MAPK) cascade, is shown to have a role in determining binding of Lck to protein partners.[68–73] Downstream of the unique region is an SH3 domain followed by an SH2 domain. These are involved in protein–protein interactions, with the SH3 domain interacting with sequences containing the core P-X-X-P motif (where P is proline and X any amino acid) while SH2 binds to phosphorylated tyrosine residues (reviewed in 74). Through a linker sequence, the SH2 domain is connected to the catalytic (SH1) region. Two tyrosine (Y) residues one within the 'activation loop' of the catalytic domain and the other at the carboxy-terminus (C-terminus) of the protein play critical regulatory roles (Y394 and Y505 in murine Lck protein).[75–78] When phosphorylated the C-terminal tyrosine interacts with the SH2

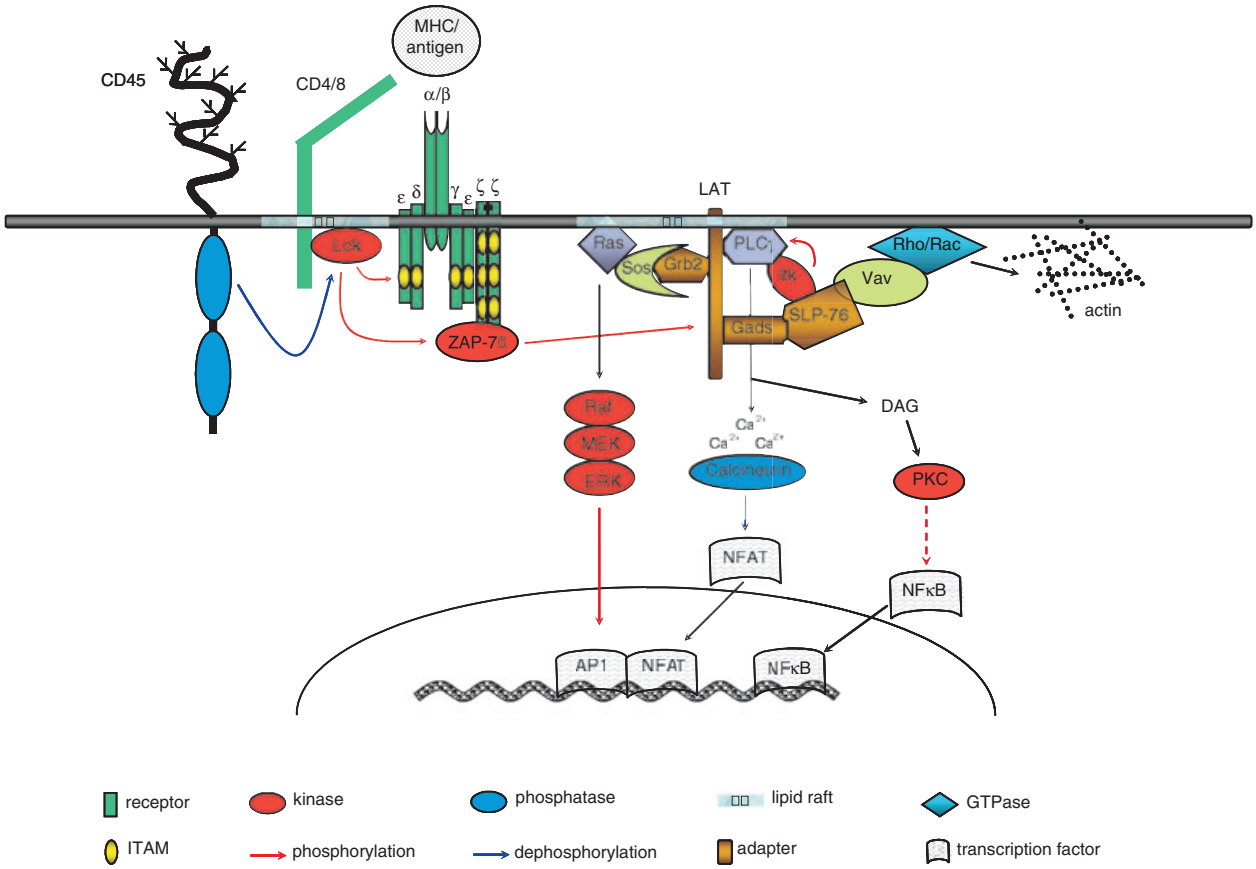

Figure 1. Signalling cascades stimulated by the TCR. Schematic depiction of key protein components of major signalling cascades that are stimulated following recognition of antigen by the TCR. Differential colouring identifies biochemical events and proteins with distinct function.

domain in the same molecule promoting the folding of the enzyme into a low activity state ('tail-bite' structure, Fig. 2).[79,80] In this configuration the SH3 domain of the protein interacts with the linker segment connecting the SH2 and SH1 modules, thus stabilizing the 'closed' structure.[78] In contrast, autophosphorylation of the tyrosine residue within the 'activation loop' induces the molecule to adopt an 'open' conformation which has significantly elevated enzymatic activity.[76,81]

The interconversion of Lck, and of other Src-family members, is dependent on the enzymatic activity of other proteins. Thus the cytosolic tyrosine kinase Csk can phosphorylate Y505 and down-regulate Lck (and other Src-family members) activity by inducing the 'tail-bite' structure (Fig. 2).[82–86] In contrast, the receptor-type tyrosine phosphatase CD45 is the principal phosphatase in T cells able to disrupt the SH2–pY505 intramolecular interaction and to cleave the phosphate group on Y505 (Fig. 2).[87–90] Interestingly, in addition to its positive role, CD45 can also downregulate the activity of Lck by dephosphorylating Y394 and possibly downstream substrates of Lck.[91,92] It is unclear how the positive and negative actions of CD45 are balanced during the early stages of TCR signalling. It is possible that the kinetics of Y505 dephosphorylation is faster providing a time window for activated Lck to phosphorylate protein substrates. Alternatively, Y505 could be more accessible to CD45 than Y394, in which case fewer phosphatase molecules located in proximity could dephosphorylate Y505 and activate Lck, while at high local concentrations of CD45 this advantage may be lost resulting in signal inhibition.

LIPID RAFT DOMAINS

Attachment of myristate and palmitate groups at the N-terminus not only promotes membrane anchorage of Lck but also governs its partitioning into lipid rafts.[58,60,63] Lipid rafts are considered as specialized microdomains within the plane of the plasma membrane with a lipid composition that is different from the glycerophospholipid-rich bilayer of the surrounding membrane. They are instead rich in glycosphingolipids, sphingomyelin and cholesterol.[93–97] One of their properties, widely used for purification purposes, is their insolubility during extraction of cells with cold non-ionic detergents, albeit different detergents may vary in their ability to solubilise lipid raft membranes.[98]

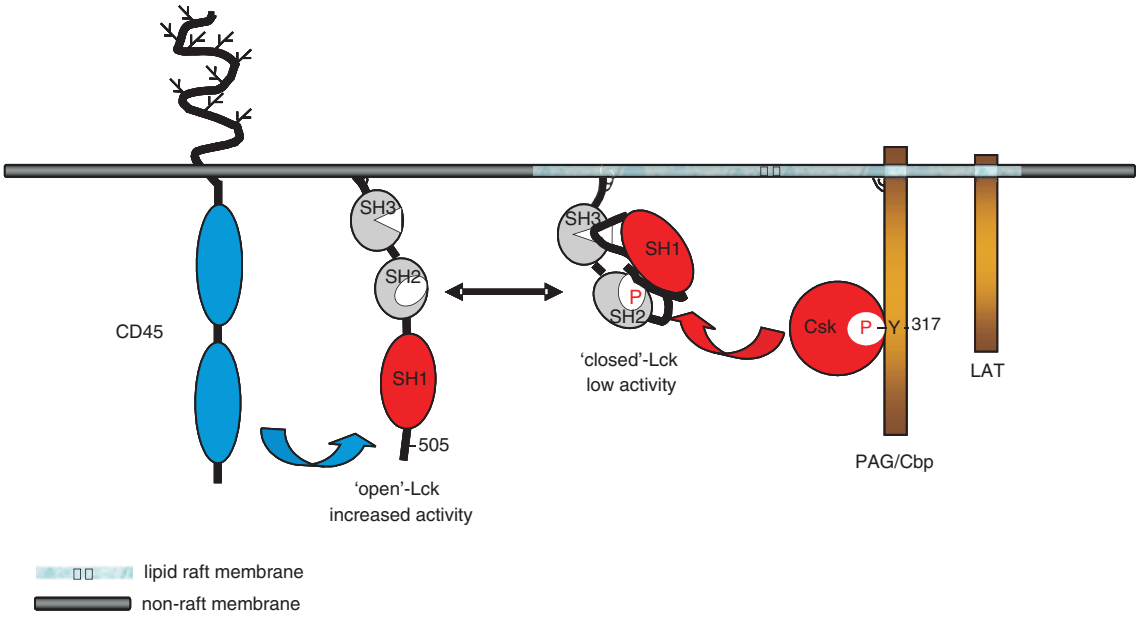

Figure 2. Regulation of Lck at the plasma membrane. In T cells, Lck is localized to both detergent-insoluble lipid rafts and detergent-soluble non-raft fractions. The raft-associated Lck is phosphorylated at the C-terminal regulatory tyrosine, which then interacts with the SH2 domain promoting a folded-low activity structure. Phosphorylation of Lck at the C-terminal tyrosine is mediated by the PAG/Cbp-Csk protein complex and it may persist in lipid rafts because of the exclusion of the CD45 tyrosine phosphatase from these domains. In contrast, the pool of Lck present in the bulk of the plasma membrane preferentially adopts an 'open' conformation with higher catalytic activity due to the positive action of CD45, which dephosphorylates the C-terminal inhibitory tyrosine.

Because of this property and their distinct lipid composition, other names given to these insoluble membranous preparations are detergent-resistant membranes (DRMs), glycosphingolipid-enriched membranes (GEMs), and detergent-insoluble glycolipid-enriched membranes (DIGs). Raft formation (or disassembly) in cell membranes could, in part, be regulated by the type and concentration of lipids present in the bilayer and in a manner similar to raft formation seen in model membranes where sphingolipids assemble to form distinct areas that are resistant to non-ionic detergent extraction.[99] Inclusion of cholesterol in these artificial lipid bilayers stabilizes the sphingolipid-formed structures.[99] Similarly, in living cells, pharmacological extraction of cholesterol from the plasma membrane results in disruption of lipid rafts, indicating that cholesterol is a critical structural component.[100–102] In the past few years, sophisticated techniques such as fluorescence resonance energy transfer (FRET),[103] fluorescence recovery after photobleaching (FRAP),[104] single particle tracking (SPT)[105,106] and chemical cross-linking,[107,108] among others, have provided support for the existence of plasma membrane domains in unperturbed cells. These techniques, by providing resolution at the nanometre scale, have suggested that lipid raft domains could be rather small structures, possibly up to 25 nm in diameter, a size much smaller from what was initially measured in detergent-insoluble preparations.[105] Therefore, detergent extraction almost certainly induces coalescence of rafts into bigger conglomerates. In addition, in intact cells it is generally assumed that lipid raft domains are not rigid structures but instead they are dynamic with lipid molecules rapidly exchanging between raft and non-raft membrane. It is unknown if *de novo* formation of lipid rafts takes place at the plasma membrane by the spontaneous assembly of resident lipids. In mammalian cells, the study of proteins which are known to target to lipid rafts revealed that incorporation of newly synthesized proteins into DRMs is first visible in the Golgi. Raft-containing vesicles subsequently move to the plasma membrane,[109] a process which may involve the actin cytoskeleton.[110] One report suggests that in T cells, lipid raft domains could be constitutively assembled by the actin cytoskeleton into larger patches, which can function as carriers for ferrying molecules to the T-cell/APC contact site during antigen presentation.[111]

Detergent insolubility and low buoyancy, which allows flotation on dense gradients, have been exploited in order to purify DRMs and to study their protein content in a variety of cell types including T lymphocytes. Initial limited analysis of proteins copurifying with the low-density, detergent-insoluble fraction indicated enrichment of signalling proteins particularly those modified by addition of lipids. Such proteins were members of the Src-family of kinases, heterotrimeric GTP-binding proteins and small GTPases, and glycosylphosphatidylinositol (GPI)-anchored receptors.[93,96] Lately, it has become apparent that a new group of signalling proteins also localises to membrane rafts. These are transmembrane adapters, which form signalling complexes at the plasma membrane. They contain two cysteine

residues as part of a C-X-X-C motif (C is cysteine and X any amino acid) that immediately follows the transmembrane segment. These membrane-proximal cysteines become palmitylated and are critical for targeting the protein to lipid rafts. Members of this group identified so far include LAT,[112] PAG/Cbp (protein associated with GEMs/Csk binding protein),[113,114] NTAL/LAB (non-T-cell activation linker/linker for activation of B cells)[115,116] and LIME (Lck-interacting molecule).[117,118] In the case of LAT, structure/function experiments have documented the importance of the C-X-X-C motif since a LAT mutant, where the two membrane-proximal cysteines were substituted, did not partition into DRMs and failed to support downstream signalling in response to TCR stimulation.[112,119]

In recent studies, proteomic analysis of purified DRM fractions was employed to produce a map of protein components associated with these domains. In the most detailed study so far published by Foster *et al.*[120] quantitative proteomics was used to specifically identify proteins whose association with the DRM from HeLa cells was sensitive to cholesterol-depleting agents. Because depletion of cholesterol disrupts lipid rafts, the authors reasoned that in contrast to contaminants resulting from the purification protocol, association of authentic raft components would be susceptible to treatment of cells with cholesterol extracting agents. Using this methodology, they identified 241 polypeptides, the majority of which were signalling proteins, but a number of structural proteins were found as well. Association of cytoskeletal proteins was also detected in detergent-insoluble rafts isolated from neutrophils.[121] Thus, this analysis supports the supposition made by earlier studies that lipid rafts may preferentially concentrate signalling molecules.

Since a functional role for lipid rafts during TCR signalling has been suggested (see section below), groups including our own have sought to identify proteins resident in DRM preparations from T cells (Fig. 3 and Table 1).[122,123] For this purpose, low density, detergent-resistant preparations from the human leukaemic T-cell line Jurkat, were resolved by one- or two-dimensional (D) gel electrophoresis and individual protein bands (or spots in the case of 2D-gels) were analysed by mass spectrometry. We resolved detergent-resistant lipid rafts isolated from 50×10^6 Jurkat cells by 2D-gel electrophoresis and proteins were visualized by silver staining (Fig. 3). Individual spots, indicated by arrows, were excised, digested with trypsin, and analysed by mass spectrometry. A list of protein spots identified by their peptide 'fingerprint' is shown in Table 1. Taken together, the results from the above studies on Jurkat lipid rafts ([122,123] and our own results) reveal an enrichment of signalling and cytoskeletal proteins in these preparations. However, the presence of mitochondrial and nuclear proteins shows that unrelated polypeptides can copurify with this method of raft preparation as there is no evidence today that nuclear and mitochondrial membranes contain microdomains. Therefore, caution should be exercised when proteins are assigned as raft-associated. On the other hand, while proteins with high affinity for raft domains are resistant to detergent extraction, molecules that are loosely associated with these domains may be sensitive to detergent extraction and therefore lost during purification. An example of weakly associated proteins whose partition to lipid rafts under certain conditions is sensitive to detergent extraction could be the TCR (see discussion below). Therefore, although detergent insolubility has been, and will continue to be, a valuable tool to study rafts and their content, more sophisticated methodologies for raft purification must be developed for the field to move forward.

Another question addressed by Bini *et al.* in their study using 2D-gel analysis was how the protein composition of DRMs changed following stimulation of the TCR. Comparison of 2D-gel protein maps corresponding to different time points of stimulation up to 15 min, showed that TCR stimulation induces substantial changes in their protein composition.[122] Intensity of some protein spots was reduced over the stimulation period, possibly indicating their exit from lipid rafts, while the silver stain signal of another group of proteins intensified indicating an increase in their affinity for raft domains.[122] These changes could reflect biological processes initiated by the stimulated TCR, which take place in membrane microdomains.

LIPID RAFTS AND TCR SIGNALLING

As mentioned above the tyrosine kinase Lck and the adapter molecule LAT constitutively reside in raft domains, a process that requires S-acylation of two membrane-proximal cysteines.[58,60,124] Mutant versions of the proteins that lack these cysteines but which remain attached to the membrane, in the case of Lck by fusion to a transmembrane domain, fail to partition into DRMs and lose their capacity to couple the TCR to downstream signalling cascades indicating that lipid raft localization is crucial for the signalling function of Lck and LAT.[63,112] Also, recently it has been suggested that following TCR stimulation Lck-containing microdomains[125] and LAT-containing microdomains[126] are recruited to the site of TCR engagement.

An ever-growing list of signalling molecules, apart from Lck and LAT, are shown to transiently translocate to membrane microdomains after stimulation of the TCR.[127–134] The CD4 coreceptor is targeted to lipid rafts through its interaction with Lck and its palmitylation on two membrane-proximal cysteine residues.[135,136] CD4 stimulation is shown to induce lipid raft aggregation and to enhance TCR signalling partly through the induction of molecular clustering at the immunological synapse.[136,137] The affinity of the TCR itself for lipid rafts seems to increase following its stimulation, as components of the TCR complex such as the ζ and ε chains and their phosphorylated/activated forms copurify with DRM fractions isolated from stimulated cells.[128,138] Recently, it was shown that T-cell activation by super-antigens is mediated by signalling events occurring in membrane microdomains.[139] In addition, confocal microscopy has revealed colocalization of TCR molecules with GPI-anchored receptors or with the ganglioside GM1, both of which are used as markers of membrane rafts.[140] GM1 is the target of cholera

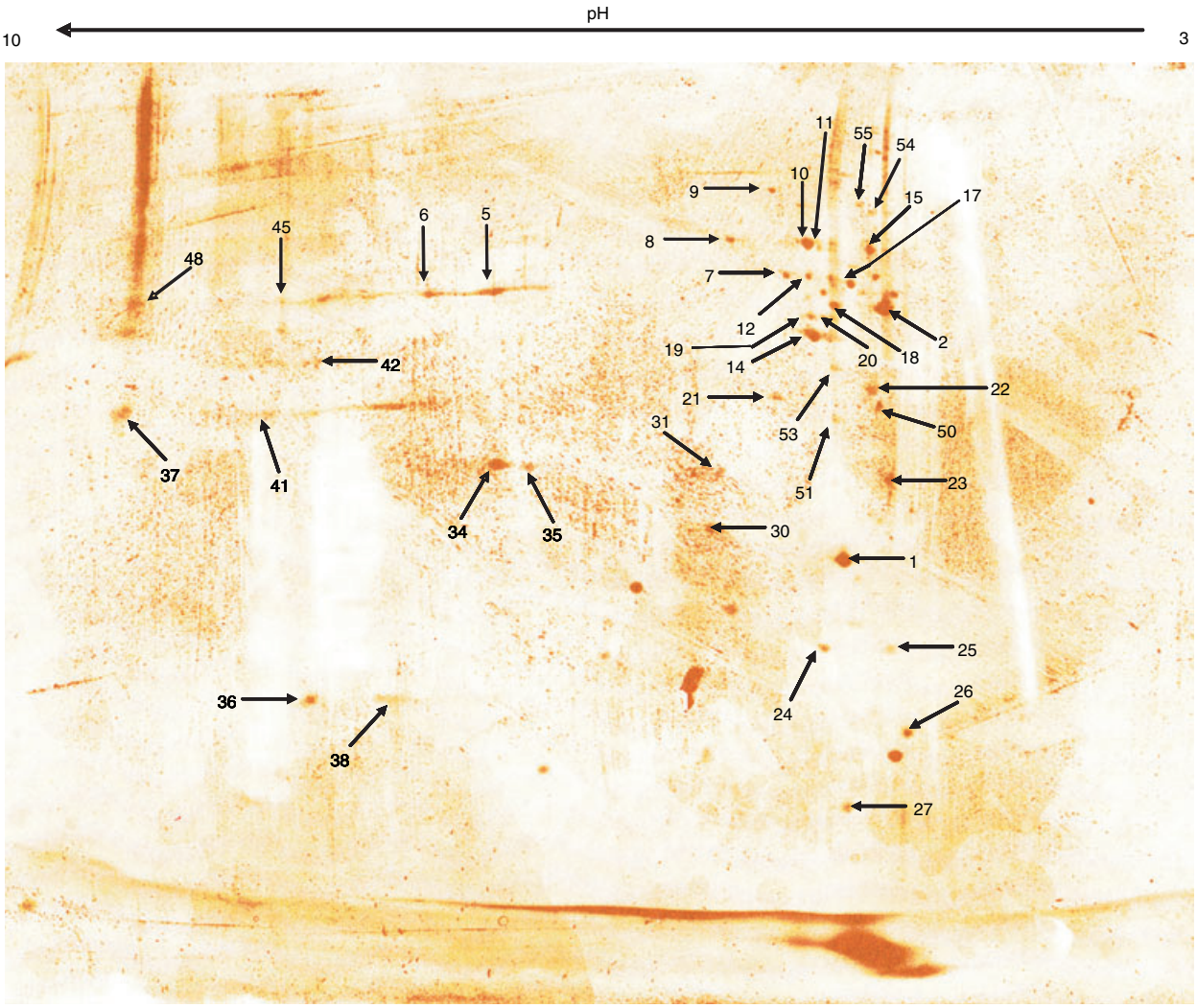

Figure 3. 2D-gel electrophoresis of T-cell lipid rafts. 50×10^6 Jurkat T cells were extracted in ice-cold lysis buffer containing 1% Triton-X-100 detergent plus protease and phosphatase inhibitors. Lipid rafts were purified by flotation on sucrose gradient and resuspended in rehydration buffer appropriate for isoelectric focusing of proteins on 3–10 pH strips. The rehydration buffer also contained 20 mM MβCD, which assists in the complete disruption of lipid rafts. Following 2D-gel electrophoresis, proteins were visualized with a silver stain compatible for analysis with mass spectrometry. Discernible protein spots were excised, digested with trypsin and the resulting peptides were recorded by mass spectrometry. The peptide 'fingerprint' obtained from this analysis was used to search available protein databases. Arrows indicate protein spots for which a positive identification was made and their identity is summarized on Table 1.

toxin B subunit (CTB) and has been extensively used in visualizing rafts and their potential colocalization with surface molecules using microscopy. However, in a recent report the authors using FRET analysis of GPI-linked proteins and CTB in Jurkat T cells were unable to detect accumulation of lipid rafts in the area of stimulated TCR complexes.[141] Furthermore, it is possible that polarization of lipid rafts during activation is T-cell subset specific since unlike $CD4^+$ T cells, primary human $CD8^+$ cells did not show polarization of lipid rafts when stimulated via their TCR and CD28 receptors.[142] How the affinity of the TCR for lipid raft domains increases upon its stimulation remains enigmatic and as of today there is no direct evidence linking induction of signalling pathways with increased affinity of the TCR for detergent-insoluble lipid rafts. Some experiments have suggested that the TCR could constitutively associate with raft domains albeit with reduced affinity. This interaction is sensitive to extraction with strong non-ionic detergents like Triton-X-100 but more resistant to mild detergents such as Brij 98.[128,140,143] Cross-linking may result in the TCR becoming more resistant to detergent extraction by increasing its affinity for lipid rafts. The cell cytoskeleton could be involved in this process.[144]

The importance of lipid rafts in TCR signalling has been suggested from experiments where T cells were treated with cholesterol-depleting agents such as methyl-β-cyclodextrin

Table 1. Proteins copurifying with detergent-insoluble membranes from T cells

Spot no.	Identified protein	MW ('000)	pI	Swiss-Prot/TrEMBL identification no.
1	Rho GDP dissociation inhibitor 2 (Rho-GDI beta)	23	5·1	P52566
2	Lymphocyte specific protein LSP1	37	4·7	P33241
5	ZAP-70 kinase (fragment)	70	7·8	P43403
6	Enolase 1α	47	7	P06733
7	Flotillin 2	42	5·2	Q14254
8	Protein disulphide isomerase ER60	57	5·9	P30101
9	Sorbin & SH3 containing protein (fragment)	100	7	Q9BX64
10	Heat-shock protein 60 (HSP60)	60	5·5	P10809
11	Transformation up-regulated nuclear protein	51	5·2	Q07244-2
12	Similar to ATP synthase, H + transporting mitochondrial F1 complex	56	5·3	P06576
14	Actin	44	5·7	P02570
15	UV excision repair protein RAD23 homologue B	43	4·8	P54727
17	ATP synthase β chain mitochondrial precursor	56	5·4	P06576
18	Dynactin 2	44	5·1	Q13561
19	Heterogenous nuclear ribonucleoprotein F	46	5·4	P52597
20	Heterogeneous nuclear ribonucleoprotein F	46	5·4	P52597
21	Capping protein	33	5·4	P47756
22	Nucleophosmin	32	4·7	P06748
23	Urokinase-type plasminogen activator receptor	32	5·8	Q03405
24	F1F0-type ATP synthase D chain	18	5·2	O75947
25	Similar to protease (prosome, macropain) 26S subunit	25	5·4	Q81V79
26	Heat shock protein (HSP60) (fragment)	60	5·5	P10809
27	Ribosomal protein S14	12	11	P06366
30	Endoplasmic reticulum lumenal protein ERp29	29	6·8	P30040
31	Telomerase reverse transcriptase (fragment)	42	5·3	Q8NG38
34	Triose phosphate isomerase	27	6·5	Q8WWDO
35	Triose phosphate isomerase	27	6·5	Q8WWDO
36	Cyclophilin B	18	8·2	P23284
37	Heterogeneous nuclear ribonucleoprotein A1	34	9·2	AAH02355
38	Cyclophilin A	18	7·7	P05092
41	Glyceraldehyde-3-phasphate dehydrogenase	36	8·3	P00354
42	Nebulette protein	82	8·5	O76041
45	C2H2 type zinc finger protein	68	8·8	O75820
48	P32/inhibitor of growth family member 1 like	33	5·1	O95698
50	Tropomyosin	30	5·1	P09493
51	Chloride intracellular channel protein 1	27	5·1	O00299
53	Aldehyde dehydrogenase 1	31	5·5	P00352
54	Haematopoietic lineage-specific protein HS1	54	4·7	P14317
55	Glucose regulated protein	72	5·1	P38646

(MβCD). Such agents disrupt raft domains and although initial reports showed inhibition of all TCR-generated signals in treated cells[138] more detailed studies subsequently revealed that such agents have more complex effects on cells by inhibiting certain signalling pathways but stimulating others.[102] Furthermore, recent work indicated that MβCD depletes intracellular Ca^{2+} stores independently of its effects on lipid raft integrity.[145] Therefore, results obtained using cholesterol-depleting agents should not be the sole supportive evidence when arguing for a role for lipid raft domains in a particular biological process.[146]

Other molecules known to participate in TCR signalling, that are transiently recruited to lipid rafts after stimulation, are ZAP-70[128,138,140] and PLCγ1.[128,138,147] Interestingly, phosphatidyl inositol 4,5 bisphosphate (PIP_2), the substrate for PLC, is enriched in DRMs suggesting that these microdomains may represent the major sites of PLC action.[148,149] Activation of PLCγ1 in lipid rafts may be facilitated by the recruitment of SLP-76[150] another adapter molecule shown to have a critical role in the regulation of downstream signalling cascades.[151] Grb2 and SOS proteins which regulate Ras activity are also recruited[45] as is the theta isoform of protein kinase C (PKCθ), which plays a critical role in T-cell activation by stimulating the NF-κB pathway.[152,153] A role for lipid rafts during costimulation has been demonstrated in T cells where CD28 engagement resulted in the redistribution of rafts to the site of TCR engagement thus amplifying and/or prolonging the TCR-generated signal.[154,155] Based on these results a model of activation has been proposed where TCR stimulation induces aggregation of rafts and phosphorylation of the receptor by resident Lck molecules, consequentially leading

to the assembly of functional 'signalosomes'. In addition to their role in costimulation, lipid raft function is regulated by the expression of negative regulators of TCR signalling, as shown for the cytotoxic T lymphocyte antigen-4 (CTLA-4) receptor. Coligation of CTLA-4 strongly inhibited the upregulation in lipid raft expression following stimulation of cells via the TCR and CD28 receptors.[155] Furthermore, a pool of CTLA-4 expressed on the surface of activated T cells is concentrated in DRM preparations where it was found to associate with the TCRζ chain, suggesting that CTLA-4 possibly functions by controlling TCR accumulation/retention in raft domains.[156,157] Collectively, these results suggest that negative regulators may limit T-cell activation by, at least in part, modifying lipid raft function. Interestingly, LAT was found to selectively associate with the open form of Lck in lipid rafts, an interaction that might have functional consequences during TCR signal transduction.[158]

Exclusion of CD45 from lipid rafts may favour tyrosine phosphorylation of protein substrates in these domains.[130,159] However, some reports have suggested that a small fraction of the phosphatase is present in DRMs and that the ectodomain of the molecule has a role in determining its membrane distribution.[160,161] Therefore, the levels of CD45 present in raft microdomains, and possibly its redistribution in and out of these domains during T-cell activation, may regulate the strength of the TCR signal by determining the levels of active Lck. Interestingly, studies on peripheral blood T cells isolated from patients with the autoimmune disease systemic lupus erythematosus, revealed that a higher proportion of CD45 associates with GM1-containing raft domains in these cells, which may be linked to their 'hyperactive' phenotype.[162–164] On the other hand, strong accumulation of CD45 in lipid rafts, as achieved experimentally by expression of a raft-targeted mutant, could have the opposite effect by inhibiting TCR signalling.[165]

Partitioning of PAG/Cbp (and possibly of LIME) to raft domains could maintain the raft-associated Lck pool in a folded inactive state in unstimulated cells (Figs 2 and 4).[102,113,158,166,167] The adapter protein PAG/Cbp is

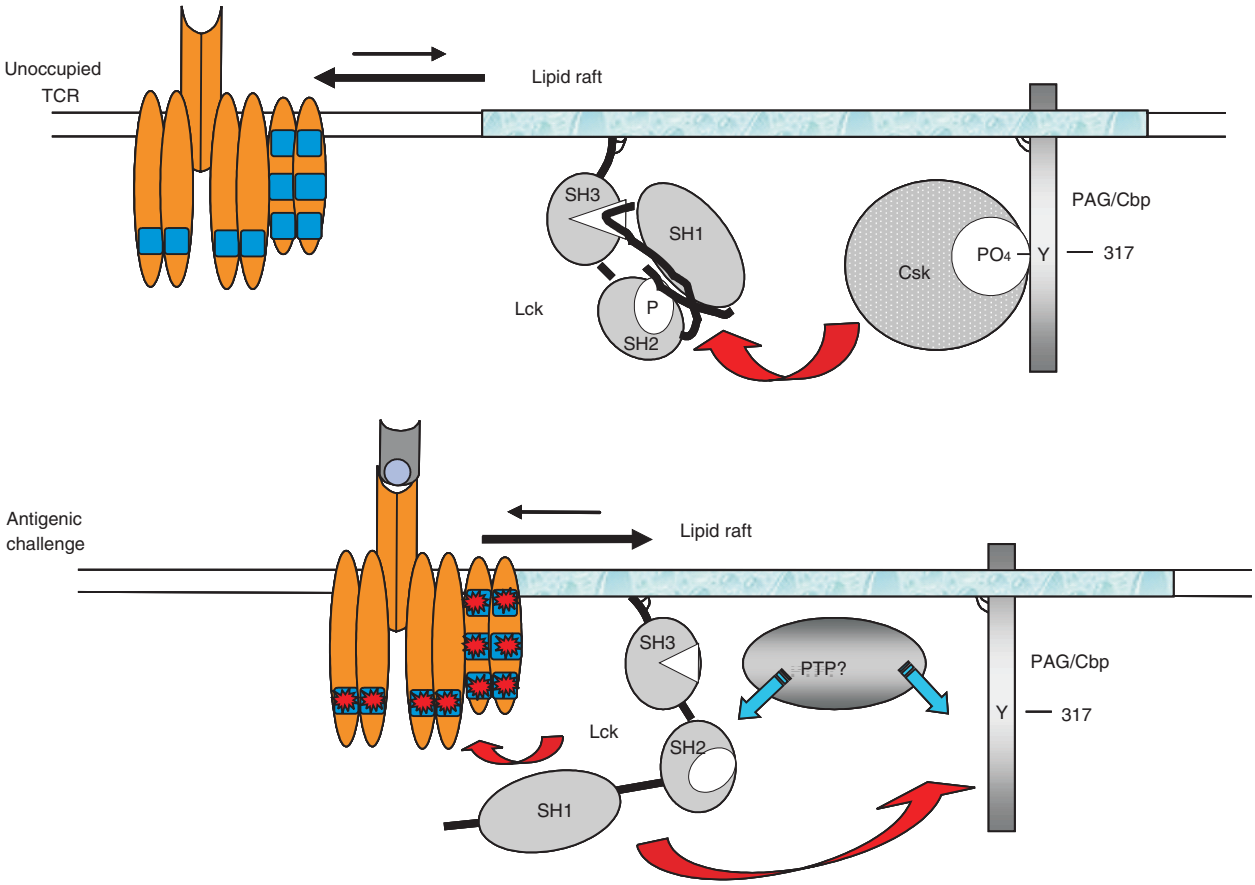

Figure 4. A two-step model for activation of the TCR in lipid rafts. In resting T cells, the TCR has low affinity for lipid raft membrane and Lck in lipid rafts is in its folded-inactive conformation due the action of the PAG/Cbp-Csk molecular complex. Antigenic stimulation of the TCR may increase its affinity for lipid rafts, a step which by itself may not be sufficient to initiate signalling. A second step may be required in which the activity of Lck in lipid rafts is transiently elevated, possibly after dephosphorylation of PAG/Cbp and dissociation of Csk, and/or dephosphorylation of Lck by a tyrosine phosphatase. Active Lck would then be able to phosphorylate the ITAMs and initiate signal transmission. Lck may also rephosphorylate PAG/Cbp leading to new recruitment of Csk and termination of the signalling cycle.

tyrosine phosphorylated in unstimulated T cells and recruits the cytosolic kinase Csk, a negative regulator of Src-family kinase activity (Fig. 2).[113,114] PAG/Cbp phosphorylation is most likely caused by the action of Fyn,[168] which is active in lipid rafts[102] and of Lck molecules that first enter raft domains from the surrounding membrane.[158] Therefore, TCR phosphorylation in lipid rafts may not only require raft aggregation but also a transient increase in the activity of raft-associated Lck. In this scenario, a tyrosine phosphatase must be involved capable of de-phosphorylating PAG/Cbp and shedding Csk from lipid rafts, and/or dephosphorylation of the inhibitory C-terminal tyrosine of Lck. Identifying this phosphatase will undoubtedly shed new light into the mechanisms of TCR signalling. Rephosphorylation of PAG/Cbp by active Lck may cause reattachment of Csk and termination of signal transduction (Fig. 4). In support of this hypothesis, it was shown that in human T cells stimulation of the TCR induces the transient dephosphorylation of PAG/Cbp and exit of Csk from raft domains.[113,169] Also, TCRζ phosphorylation and NF-AT production was increased in Jurkat T cells expressing dominant-negative Csk mutants.[169,170] Interestingly, in murine $CD4^+$ T cells, it was shown that cross-linking of the TCR with CD4 rapidly induces the activity and subsequent translocation of a small fraction of Lck from detergent-soluble to detergent-resistant membrane. This was followed by an increase in the activity of Fyn residing in DRMs, suggesting cross-regulation of these two kinases in raft domains.[171,172] This transient increase of Src activity in raft membrane could facilitate activation of the TCR but in addition, increased Fyn activity may assist in reformation of the PAG/Cbp-Csk inhibitory complex.

CONCLUSIONS AND FUTURE CONSIDERATIONS

In the past few years, membrane microdomains have become a popular subject of study across many disciplines. A substantial volume of work, which includes functional experiments and proteomics analysis, points to an important role for these domains as regulators of signal transduction pathways in lymphocytes. Their importance in signalling most likely reflects their ability to compartmentalise proteins at the plasma membrane and upon receptor stimulation to facilitate the assembly of signalling complexes ('signalosomes'). However, despite the substantial progress made so far, critical questions remain unanswered. Hence, the structure of lipid rafts remains elusive, as is potential changes in their size and protein/lipid composition during stimulation or through the different stages of cell differentiation. One approach that can potentially provide useful information could be the systematic analysis of detergent-resistant membrane preparations using proteomics. Such an analysis could reveal which proteins and when move in and out of rafts during receptor signalling, and in the case of T cells during TCR stimulation. Also, such analysis could potentially identify post-translational modifications (i.e. phosphorylation, lipidation, ubiquitination) of DRM-associated proteins induced by receptor stimulation, which in certain cases may be indicative of the signalling activity of the protein. Information assimilated from the proteomics analysis can form the basis for constructing a 'map of events' taking place in lipid rafts after TCR stimulation.

It is also unknown if lipid rafts represent a homogeneous population or whether different types of rafts exist, potentially performing distinct tasks. Studies in leucocytes suggest that structurally and functionally diverse membrane domains may exist with a role in determining rear-front polarity during cell movement.[173–176] Further progress in this area will critically depend upon the development of new methods, as well as in the identification of specific raft markers which will allow us to visualize and track lipid rafts in living cells and possibly discriminate between different subtypes of microdomains. Understanding in detail how lipid rafts operate in T cells will not only refine our current theories of how TCR transduces signals, but will undoubtedly have implications in other fields of biology as well.

ACKNOWLEDGMENTS

This work was supported by a Wellcome Trust Career Development Award to P. S. Kabouridis (ref. no. 58408) and by the Joint Research Board of the Queen Mary's School of Medicine & Dentistry. E. C. Jury is supported by the Arthritis Research Campaign. We thank Yuti Chernajovsky for critically reading the manuscript.

REFERENCES

1 Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 1996; **384:**134–41.
2 Davis MM, Boniface JJ, Reich Z, Lyons D, Hampl J, Arden B, Chien Y. Ligand recognition by alpha beta T cell receptors. Annu Rev Immunol 1998; **16:**523–44.
3 Wange RL, Samelson LE. Complex complexes: signaling at the TCR. Immunity 1996; **5:**197–205.
4 Qian D, Weiss A. T cell antigen receptor signal transduction. Curr Opin Cell Biol 1997; **9:**205–12.
5 Werlen G, Palmer E. The T-cell receptor signalosome: a dynamic structure with expanding complexity. Curr Opin Immunol 2002; **14:**299–305.
6 Reth M. Antigen receptor tail clue. Nature 1989; **338:**383–4.
7 Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor ζ chain is sufficient to couple to receptor-associated signal transduction pathways. Cell 1991; **64:**891–901.
8 Irving BA, Chan AC, Weiss A. Functional characterization of a signal transducing motif present in the T cell antigen receptor zeta chain. J Exp Med 1993; **177:**1093–103.
9 Cambier JC. Antigen and Fc receptor signaling. The awesome power of the immunoreceptor tyrosine-based activation motif (ITAM). J Immunol 1995; **155:**3281–5.
10 Ardouin L, Boyer C, Gillet A *et al*. Crippling of CD3-zeta ITAMs does not impair T cell receptor signaling. Immunity 1999; **10:**409–20.
11 Love PE, Shores EW. ITAM multiplicity and thymocyte selection: how low can you go? Immunity 2000; **12:**591–7.

12 Germain RN, Stefanova I. The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation. Annu Rev Immunol 1999; **17:**467–522.
13 Cantrell D. T cell antigen receptor signal transduction pathways. Annu Rev Immunol 1996; **14:**259–74.
14 Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell 1994; **76:**263–74.
15 van Leeuwen JEM, Samelson LE. T cell antigen-receptor signal transduction. Curr Opin Immunol 1999; **11:**242–8.
16 Latour S, Veillette A. Proximal protein tyrosine kinases in immunoreceptor signaling. Curr Opin Immunol 2001; **13:**299–306.
17 Straus DB, Weiss A. Genetic evidence for the involvement of the lck tyrosine kinase in signal transduction through the T cell antigen receptor. Cell 1992; **70:**585–93.
18 Karnitz L, Sutor SL, Toshihiko T, Reed JC, Bell MP, McKean DJ, Leibson PJ, Abraham RT. Effects of p56lck deficiency on the growth and cytolytic effector function of an interleukin-2 dependent cytotoxic T cell line. Mol Cell Biol 1992; **12:**4521–30.
19 Molina TJ, Kishihara K, Siderovski DP *et al*. Profound block in thymocyte development in mice lacking p56lck. Nature 1992; **357:**161–4.
20 Seddon B, Legname G, Tomlinson P, Zamoyska R. Long-term survival but impaired homeostatic proliferation of naive T cells in the absence of p56lck. Science 2000; **290:**127–31.
21 Rudd CE, Trevillyan JM, Dasgupta JD, Wong LL, Schlossman SF. The CD4 receptor is complexed in detergent lysates to a protein-tyrosine kinase (pp58) from human T lymphocytes. Proc Natl Acad Sci USA 1988; **85:**5190–4.
22 Veillette A, Bookman MA, Horak EM, Bolen JB. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck. Cell 1988; **55:**301–8.
23 Barber EK, Dasgupta JD, Schlossman SF, Trevillyan JM, Rudd CE. The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56lck) that phosphorylates the CD3 complex. Proc Natl Acad Sci USA 1989; **86:**3277–81.
24 Stein PL, Lee H-M, Rich S, Soriano P. pp59fyn mutant mice display differential signaling in thymocytes and peripheral T cells. Cell 1992; **70:**741–50.
25 Groves T, Smiley P, Cooke MP, Forbush K, Perlmutter RM, Guidos CJ. Fyn can partially substitute for Lck in T lymphocyte development. Immunity 1996; **5:**417–28.
26 da Silva AJ, Li Z, de Vera C, Canto E, Findell P, Rudd CE. Cloning of a novel T-cell protein FYB that binds FYN and SH2-domain-containing leukocyte protein 76 and modulates interleukin 2 production. Proc Natl Acad Sci USA 1997; **94:**7493–8.
27 Musci MA, Hendricks-Taylor LR, Motto DG, Paskind M, Kamens J, Turck CW, Koretzky GA. Molecular cloning of SLAP-130, an SLP-76-associated substrate of the T cell antigen receptor-stimulated protein tyrosine kinases. J Biol Chem 1997; **272:**11674–7.
28 Marie-Cardine A, Bruyns E, Eckerskorn C, Kirchgessner H, Meuer SC, Schraven B. Molecular cloning of SKAP55, a novel protein that associates with the protein tyrosine kinase p59fyn in human T-lymphocytes. J Biol Chem 1997; **272:**16077–80.
29 Griffiths EK, Krawczyk C, Kong YY *et al*. Positive regulation of T cell activation and integrin adhesion by the adapter Fyb/Slap. Science 2001; **293:**2260–3.
30 Peterson EJ, Woods ML, Dmowski SA *et al*. Coupling of the TCR to integrin activation by Slap-130/Fyb. Science 2001; **293:**2263–5.
31 Wang H, Moon EY, Azouz A, Wu X, Smith A, Schneider H, Hogg N, Rudd CE. SKAP-55 regulates integrin adhesion and formation of T cell-APC conjugates. Nat Immunol 2003; **4:**366–74.
32 Wu LYuZ, Shen SH. SKAP55 recruits to lipid rafts and positively mediates the MAPK pathway upon T cell receptor activation. J Biol Chem 2002; **277:**40420–7.
33 Chan AC, Iwashima M, Turck CW, Weiss A. ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. Cell 1992; **71:**649–62.
34 Chan AC, Dalton M, Johnson R, Kong GH, Wang T, Thoma R, Kurosaki T. Activation of ZAP-70 kinase activity by phosphorylation of tyrosine 493 is required for lymphocyte antigen receptor function. EMBO J 1995; **14:**2499–508.
35 Wange RL, Guitian R, Isakov N, Watts JD, Aebersold R, Samelson LE. Activating and inhibitory mutations in adjacent tyrosines in the kinase domain of ZAP-70. J Biol Chem 1995; **270:**18730–3.
36 Chan AC, Shaw AS. Regulation of antigen receptor signal transduction by protein tyrosine kinases. Curr Opin Immunol 1996; **8:**394–401.
37 Arpaia E, Shahar M, Dadi H, Cohen A, Roifman CM. Defective T cell receptor signaling and $CD8^+$ thymic selection in humans lacking zap-70 kinase. Cell 1994; **76:**947–58.
38 Elder ME, Lin D, Clever J, Chan AC, Hope TJ, Weiss A, Parslow TG. Human severe combined immunodeficiency due to a defect in ZAP-70, a T cell tyrosine kinase. Science 1994; **264:**1596–9.
39 Chan AC, Kadlecek TA, Elder ME, Filipovich AH, Kuo WL, Iwashima M, Parslow TG, Weiss A. ZAP-70 deficiency in an autosomal recessive form of severe combined immunodeficiency. Science 1994; **264:**1599–601.
40 Negishi I, Motoyama N, Nakayama K *et al*. Essential role for ZAP-70 in both positive and negative selection of thymocytes. Nature 1995; **376:**435–8.
41 Zhang W, Sloan-Lancaster J, Kitchen J, Trible RP, Samelson LELAT. The ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. Cell 1998; **92:**83–92.
42 Wange RL. LAT, the linker for activation of T cells. A bridge between T cell-specific and general signaling pathways. Science's STKE 2000; www.stke.org/cgi/content/full/OC_sigtrans; 2000/63/re:1–13 (web only).
43 Harder T, Kuhn M. Selective accumulation of raft-associated membrane protein LAT in T cell receptor signaling assemblies. J Cell Biol 2000; **151:**199–208.
44 Samelson LA. Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. Annu Rev Immunol 2002; **20:**371–94.
45 Finco TS, Kadlecek T, Zhang W, Samelson LE, Weiss A. LAT is required for TCR-mediated activation of PLCg1 and the Ras pathway. Immunity 1998; **9:**617–26.
46 Zhang W, Sommers CL, Burshtyn DN *et al*. Essential role of LAT in T cell development. Immunity 1999; **10:**323–32.
47 Liu SK, Fang N, Koretzky GA, McGlade CJ. The hematopoietic-specific adaptor protein gads functions in T-cell signaling via interactions with the SLP-76 and LAT adaptors. Curr Biol 1999; **9:**67–75.
48 Sommers CL, Rabin RL, Grinberg A, Tsay HC, Farber J, Love PE. A role for the Tec family tyrosine kinase Txk in T cell activation and thymocyte selection. J Exp Med 1999; **190:**1427–38.
49 Ching KA, Grasis JA, Tailor P, Kawakami Y, Kawakami T, Tsoukas CD. TCR/CD3-induced activation and binding of

Emt/Itk to linker of activated T cell complexes: requirement for the Src homology 2 domain. J Immunol 2000; **165:**256–62.
50 Yablonski D, Kuhne MR, Kadlecek T, Weiss A. Uncoupling of nonreceptor tyrosine kinases from PLC-gamma1 in an SLP-76-deficient T cell. Science 1998; **281:**413–6.
51 Cantrell DA. GTPases and T cell activation. Immunol Rev 2003; **192:**122–30.
52 Acuto O, Cantrell D. T cell activation and the cytoskeleton. Annu Rev Immunol 2000; **18:**165–84.
53 Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 1997; **13:**513–609.
54 Sicheri F, Kuriyan J. Structures of Src-family tyrosine kinases. Curr Opin Struct Biol 1997; **7:**777–85.
55 Resh MD. Myristylation and palmitylation of Src family members: the fats of the matter. Cell 1994; **76:**411–3.
56 Jackson CS, Zlatkine P, Bano C *et al*. Dynamic protein acylation and the regulation of localization and function of signal-transducing proteins. Biochem Soc Trans 1995; **23:**568–71.
57 Milligan G, Parenti M, Magee AI. The dynamic role of palmitoylation in signal transduction. Trends Biochem Sci 1995; **20:**181–7.
58 Shenoy-Scaria AM, Gauen LKT, Kwong J, Shaw AS, Lublin DM. Palmitylation of an amino-terminal cysteine motif of protein tyrosine kinases p56lck and p59fyn mediates interaction with glycosyl-phosphatidylinositol-anchored proteins. Mol Cell Biol 1993; **13:**6385–92.
59 Shenoy-Scaria AM, Dietzen DJ, Kwong J, Link DC, Lublin DM. Cysteine3 of Src family protein tyrosine kinases determines palmitoylation and localization in caveolae. J Cell Biol 1994; **126:**353–63.
60 Rodgers W, Crise B, Rose JK. Signals determining protein tyrosine kinase and glycosyl-phosphatidylinositol-anchored protein targeting to a glycolipid-enriched membrane fraction. Mol Cell Biol 1994; **14:**5384–91.
61 Koegl M, Zlatkine P, Ley SC, Courtneidge SA, Magee AI. Palmitoylation of multiple Src-family kinases at a homologous N-terminal motif. Biochem J 1994; **303:**749–53.
62 Timson Gauen LK, Linder ME, Shaw AS. Multiple features of the p59fyn src homology 4 domain define a motif for immune-receptor tyrosine-based activation motif (ITAM) binding and for plasma membrane localization. J Cell Biol 1996; **133:**1007–15.
63 Kabouridis PS, Magee AI, Ley SC. S-acylation of LCK protein tyrosine kinase is essential for its signalling function in T lymphocytes. EMBO J 1997; **16:**4983–98.
64 Bijlmakers MJ, Isobe-Nakamura M, Ruddock LJ, Marsh M. Intrinsic signals in the unique domain target p56 (lck) to the plasma membrane independently of CD4. J Cell Biol 1997; **137:**1029–40.
65 van't Hof W, Resh MD. Rapid plasma membrane anchoring of newly synthesized p59fyn: selective requirement for NH2-terminal myristoylation and palmitoylation at cysteine-3. J Cell Biol 1997; **136:**1023–35.
66 Kwong J, Lublin DM. Amino-terminal palmitate or polybasic domain can provide required second signal to myristate for membrane binding of p56lck. Biochem Biophys Res Commun 1995; **207:**868–76.
67 Zlatkine P, Mehul B, Magee AI. Retargeting of cytosolic proteins to the plasma membrane by the Lck protein tyrosine kinase dual acylation motif. J Cell Sci 1997; **110:**673–9.
68 Watts JD, Sanghera JS, Pelech SL, Aebersold R. Phosphorylation of serine 59 of p56lck in activated T cells. J Biol Chem 1993; **268:**23275–82.
69 Winkler DG, Park I, Kim T, Payne NS, Walsh CT, Strominger JL, Shin J. Phosphorylation of Ser-42 and Ser-59 in the N-terminal region of the tyrosine kinase p56lck. Proc Natl Acad Sci USA 1993; **90:**5176–80.
70 Gervais FG, Veillette A. The unique amino-terminal domain of p56lck regulates interactions with tyrosine protein phosphatases in T lymphocytes. Mol Cell Biol 1995; **15:**2393–401.
71 Joung I, Kim T, Stolz LA, Payne G, Winkler DG, Walsh CT, Strominger JL, Shin J. Modification of Ser59 in the unique N-terminal region of tyrosine kinase p56lck regulates specificity of its Src homology 2 domain. Proc Natl Acad Sci USA 1995; **92:**5778–82.
72 Park I, Chung J, Walsh CT, Yun Y, Strominger JL, Shin J. Phosphotyrosine-independent binding of a 62-kDa protein to the src homology 2 (SH2) domain of p56lck and its regulation by phosphorylation of Ser-59 in the lck unique N-terminal region. Proc Natl Acad Sci USA 1995; **92:**12338–42.
73 Kesavan KP, Isaacson CC, Ashendel CL, Geahlen RL, Harrison ML. Characterization of the *in vivo* sites of serine phosphorylation on Lck identifying serine 59 as a site of mitotic phosphorylation. J Biol Chem 2002; **277:**14666–73.
74 Pawson T, Nash P. Assembly of cell regulatory systems through protein interaction domains. Science 2003; **300:**445–52.
75 Reynolds AB, Vila J, Lansing TJ, Potts WM, Weber MJ, Parsons JT. Activation of the oncogenic potential of the avian cellular src protein by specific structural alteration of the carboxy terminus. EMBO J 1987; **6:**2359–64.
76 MacAuley A, Cooper JA. Structural defferences between repressed and derepressed forms of p60c-src. Mol Cell Biol 1989; **9:**2648–56.
77 Reynolds PJ, Hurley TR, Sefton BM. Functional analysis of the SH2 and SH3 domains of the lck tyrosine protein kinase. Oncogene 1992; **7:**1949–55.
78 Moarefi I, LaFevre-Bernt M, Sicheri F, Huse M, Lee CH, Kuriyan J, Miller WT. Activation of the Src-family tyrosine kinase Hck by SH3 domain displacement. Nature 1997; **385:**650–3.
79 Xu W, Harrison SC, Eck MJ. Three-dimensional structure of the tyrosine kinase c-Src. Nature 1997; **385:**595–602.
80 Xu W, Doshi A, Lei M, Eck MJ, Harrison SC. Crystal structures of c-Src reveal features of its autoinhibitory mechanism. Molec Cell 1999; **3:**629–38.
81 Yamaguchi H, Hendrickson WA. Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation. Nature 1996; **384:**484–9.
82 Okada M, Nakagawa H. A protein tyrosine kinase involved in regulation of pp60c-src function. J Biol Chem 1989; **264:**20886–93.
83 Nada S, Okada M, MacAuley A, Cooper JA, Nakagawa H. Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60c-src. Nature 1991; **351:**69–72.
84 Okada M, Nada S, Yamanashi Y, Yamamoto T, Nakagawa HCSK. a protein-tyrosine kinase involved in regulation of src family kinases. J Biol Chem 1991; **266:**24249–52.
85 Bergman M, Mustelin T, Oetken C *et al*. The human p50csk tyrosine kinase phosphorylates p56lck at Tyr-505 and down regulates its catalytic activity. EMBO J 1992; **11:**2919–24.
86 Chow LM, Fournel M, Davidson D, Veillette A. Negative regulation of T-cell receptor signalling by tyrosine protein kinase p50csk. Nature 1993; **365:**156–60.
87 Mustelin T, Altman A. Dephosphorylation and activation of the T cell tyrosine kinase pp56lck by the leukocyte common antigen (CD45). Oncogene 1990; **5:**809–13.

88 Sieh M, Bolen JB, Weiss A. CD45 specifically modulates binding of Lck to a phosphopeptide encompassing the negative regulatory tyrosine of Lck. EMBO J 1993; **12:**315–21.
89 Burns CM, Sakaguchi K, Appella E, Ashwell JD. CD45 regulation of tyrosine phosphorylation and enzyme activity of src family kinases. J Biol Chem 1994; **269:**13594–600.
90 Penninger JM, Irie-Sasaki J, Sasaki T, Oliveira-dos-Santos AJ. CD45: new jobs for an old acquaintance. Nat Immunol 2001; **2:**389–96.
91 D'Oro U, Ashwell JD. Cutting edge. the CD45 tyrosine phosphatase is an inhibitor of Lck activity in thymocytes. J Immunol 1999; **162:**1879–83.
92 Thomas ML, Brown EJ. Positive and negative regulation of Src-family membrane kinases by CD45. Immunol Today 1999; **20:**406–11.
93 Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997; **387:**569–72.
94 Harder T, Simons K. Caveolae, DIGs, and the dynamics of sphingolipid-cholesterol microdomains. Curr Opin Cell Biol 1997; **9:**534–42.
95 Edidin M. Lipid microdomains in cell surface membranes. Curr Opin Struct Biol 1997; **7:**528–32.
96 Brown DA, London E. Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 1998; **14:**111–36.
97 Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 2000; **1:**31.
98 Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K. Resistance of cell membranes to different detergents. Proc Natl Acad Sci USA 2003; **100:**5795–800.
99 Brown RE. Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. J Cell Sci 1998; **111:**1–9.
100 Furuchi T, Anderson RGW. Cholesterol depletion of caveolae causes hyperactivation of extracellular signal-related kinase (ERK). J Biol Chem 1998; **273:**21099–104.
101 Visconti PE, Ning X, Fornes MW, Alvarez JG, Stein P, Connors SA, Kopf GS. Cholesterol efflux-mediated signal transduction in mammalian sperm: cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation. Dev Biol 1999; **214:**429–43.
102 Kabouridis PS, Janzen J, Magee AL, Ley SC. Cholesterol depletion disrupts lipid rafts and modulates the activity of multiple signaling pathways in T lymphocytes. Eur J Immunol 2000; **30:**954–63.
103 Zacharias DA, Violin JD, Newton AC, Tsien RY. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science 2002; **296:**913–6.
104 Shvartsman DE, Kotler M, Tall RD, Roth MG, Henis YI. Differently anchored influenza hemagglutinin mutants display distinct interaction dynamics with mutual rafts. J Cell Biol 2003; **163:**879–88.
105 Pralle A, Keller P, Florin EL, Simons K, Horber JK. Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. J Cell Biol 2000; **148:**997–1008.
106 Subczynski WK, Kusumi A. Dynamics of raft molecules in the cell and artificial membranes. approaches by pulse EPR spin labeling and single molecule optical microscopy. Biochim Biophys Acta 2003; **1610:**231–43.
107 Friedrichson T, Kurzchalia TV. Microdomains of GPI-anchored proteins in living cells revealed by crosslinking. Nature 1998; **394:**356–60.
108 Varma R, Mayor S. GPI-anchored proteins are organized in submicron domains at the cell surface. Nature 1998; **394:**798–801.
109 Brown DA, London E. Structure and origin of ordered lipid domains in biological membranes. J Membr Biol 1998; **164:**103–14.
110 Rozelle AL, Machesky LM, Yamamoto M *et al*. Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3. Curr Biol 2000; **10:**311–20.
111 Jordan S, Rodgers W. T cell glycolipid-enriched membrane domains are constitutively assembled as membrane patches that translocate to immune synapses. J Immunol 2003; **171:**78–87.
112 Zhang W, Trible RP, Samelson LE. LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity 1998; **9:**239–46.
113 Brdicka T, Pavlistova D, Leo A *et al*. Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. J Exp Med 2000; **191:**1591–604.
114 Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K, Tarakhovsky A, Okada M. Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. Nature 2000; **404:**999–1003.
115 Brdicka T, Imrich M, Angelisova P *et al*. Non-T cell activation linker (NTAL): a transmembrane adaptor protein involved in immunoreceptor signaling. J Exp Med 2002; **196:**1617–26.
116 Janssen E, Zhang W. Adaptor proteins in lymphocyte activation. Curr Opin Immunol 2003; **15:**269–76.
117 Brdickova N, Brdicka T, Angelisova P *et al*. LIME. A new membrane raft-associated adaptor protein involved in CD4 and CD8 coreceptor signaling. J Exp Med 2003; **198:**1453–62.
118 Hur EM, Son M, Lee OH, Choi YB, Park C, Lee H, Yun Y. LIME, a novel transmembrane adaptor protein, associates with p56lck and mediates T cell activation. J Exp Med 2003; **198:**1463–73.
119 Lin J, Weiss A, Finco TS. Localization of LAT in glycolipid-enriched microdomains is required for T cell activation. J Biol Chem 1999; **274:**28861–4.
120 Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci USA 2003; **100:**5813–8.
121 Nebl T, Pestonjamasp KN, Leszyk JD, Crowley JL, Oh SW, Luna EJ. Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. J Biol Chem 2002; **277:**43399–409.
122 Bini L, Pacini S, Liberatori S, Valensin S, Pellegrini M, Raggiaschi R, Pallini V, Baldari CT. Extensive temporally regulated reorganization of the lipid raft proteome following T-cell antigen receptor triggering. Biochem J 2003; **369:**301–9.
123 von Haller PD, Donohoe S, Goodlett DR, Aebersold R, Watts JD. Mass spectrometric characterization of proteins extracted from Jurkat T cell detergent-resistant membrane domains. Proteomics 2001; **1:**1010–21.
124 Brdicka T, Cerny J, Horejsi V. T cell receptor signalling results in rapid tyrosine phosphorylation of the linker protein LAT present in detergent-resistant membrane microdomains. Biochem Biophys Res Commun 1998; **248:**356–60.
125 Ike H, Kosugi A, Kato A, Iino R, Hirano H, Fujiwara T, Ritchie K, Kusumi A. Mechanism of Lck recruitment to the T-cell receptor cluster as studied by single-molecule-fluorescence video imaging. Chemphyschem 2003; **4:**620–6.

126 Tanimura N, Nagafuku M, Minaki Y *et al*. Dynamic changes in the mobility of LAT in aggregated lipid rafts upon T cell activation. J Cell Biol 2003; **160:**125–35.
127 Xavier R, Seed B. Membrane compartmentation and the response to antigen. Curr Opin Immunol 1999; **11:**265–9.
128 Montixi C, Langlet C, Bernard AM *et al*. Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. EMBO J 1998; **17:**5334–48.
129 Moran M, Miceli MC. Engagement of GPI-linked CD48 contributes to TCR signals and cytoskeletal reorganization: a role for lipid rafts in T cell activation. Immunity 1998; **9:**787–96.
130 Janes PW, Ley SC, Magee AI, Kabouridis PS. The role of lipid rafts in T cell antigen receptor (TCR) signalling. Semin Immunol 2000; **12:**23–34.
131 Langlet C, Bernard AM, Drevot P, He HT. Membrane rafts and signaling by the multichain immune recognition receptors. Curr Opin Immunol 2000; **12:**250.
132 Viola A. The amplification of TCR signaling by dynamic membrane microdomains. Trends Immunol 2001; **22:**322–7.
133 Leitenberg D, Balamuth F, Bottomly K. Changes in the T cell receptor macromolecular signaling complex and membrane microdomains during T cell development and activation. Semin Immunol 2001; **13:**129–38.
134 Johmura S, Oh-hora M, Inabe K *et al*. Regulation of Vav localization in membrane rafts by adaptor molecules Grb2 and BLNK. Immunity 2003; **18:**777–87.
135 Foti M, Phelouzat MA, Holm A, Rasmusson BJ, Carpentier JL. p56Lck anchors CD4 to distinct microdomains on microvilli. Proc Natl Acad Sci USA 2002; **99:**2008–13.
136 Fragoso R, Ren D, Zhang X, Su MW, Burakoff SJ, Jin YJ. Lipid raft distribution of CD4 depends on its palmitoylation and association with Lck, and evidence for CD4-induced lipid raft aggregation as an additional mechanism to enhance CD3 signaling. J Immunol 2003; **170:**913–21.
137 Balamuth F, Brogdon JL, Bottomly K. CD4 raft association and signaling regulate molecular clustering at the immunological synapse site. J Immunol 2004; **172:**5887–92.
138 Xavier R, Brennan T, Li Q, McCormack C, Seed B. Membrane compartmentation is required for efficient T cell activation. Immunity 1998; **8:**356–60.
139 Pizzo P, Giurisato E, Bigsten A, Tassi M, Tavano R, Shaw A, Viola A. Physiological T cell activation starts and propagates in lipid rafts. Immunol Lett 2004; **91:**3–9.
140 Janes PW, Ley SC, Magee AI. Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. J Cell Biol 1999; **147:**447–61.
141 Glebov OO, Nichols BJ. Lipid raft proteins have a random distribution during localized activation of the T-cell receptor. Nat Cell Biol 2004; **6:**238–43.
142 Kovacs B, Maus MV, Riley JL, Derimanov GS, Koretzky GA, June CH, Finkel TH. Human $CD8^+$ T cells do not require the polarization of lipid rafts for activation and proliferation. Proc Natl Acad Sci USA 2002; **99:**15006–11.
143 Drevot P, Langlet C, Guo XJ, Bernard AM, Colard O, Chauvin JP, Lasserre R, He HT. TCR signal initiation machinery is pre-assembled and activated in a subset of membrane rafts. EMBO J 2002; **21:**1899–908.
144 Harder T, Simons K. Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation. Eur J Immunol 1999; **29:**556–62.
145 Pizzo P, Giurisato E, Tassi M, Benedetti A, Pozzan T, Viola A. Lipid rafts and T cell receptor signaling: a critical re-evaluation. Eur J Immunol 2002; **32:**3082–91.
146 Pizzo P, Viola A. Lymphocyte lipid rafts: structure and function. Curr Opin Immunol 2003; **15:**255–60.
147 Veri MC, DeBell KE, Seminario MC *et al*. Membrane raft-dependent regulation of phospholipase Cgamma-1 activation in T lymphocytes. Mol Cell Biol 2001; **21:**6939–50.
148 Hope HR, Pike LJ. Phosphoinositides and phosphoinositide-utilizing enzymes in detergent-insoluble lipid domains. Mol Biol Cell 1996; **7:**843–51.
149 Liu Y, Casey L, Pike LJ. Compartmentalization of phosphatidylinositol 4,5-bisphosphate in low-density membrane domains in the absence of caveolin. Biochem Biophys Res Commun 1998; **245:**684–90.
150 Boerth NJ, Sadler JJ, Bauer DE, Clements JL, Gheith SM, Koretzky GA. Recruitment of SLP-76 to the membrane and glycolipid-enriched membrane microdomains replaces the requirement for linker for activation of T cells in T cell receptor signaling. J Exp Med 2000; **192:**1047–58.
151 Jordan MS, Singer AL, Koretzky GA. Adaptors as central mediators of signal transduction in immune cells. Nat Immunol 2003; **4:**110–6.
152 Khoshnan A, Bae D, Tindell CA, Nel AE. The physical association of protein kinase C theta with a lipid raft-associated inhibitor of kappa B factor kinase (IKK) complex plays a role in the activation of the NF-kappa B cascade by TCR and CD28. J Immunol 2000; **165:**6933–40.
153 Bi K, Tanaka Y, Coudronniere N, Sugie K, Hong S, van Stipdonk MJ, Altman A. Antigen-induced translocation of PKC-theta to membrane rafts is required for T cell activation. Nat Immunol 2001; **2:**556–63.
154 Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A. T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science 1999; **283:**680–2.
155 Martin M, Schneider H, Azouz A, Rudd CE. Cytotoxic T lymphocyte antigen 4 and CD28 modulate cell surface raft expression in their regulation of T-cell function. J Exp Med 2001; **194:**1675–81.
156 Chikuma S, Imboden JB, Bluestone JA. Negative regulation of T cell receptor–lipid raft interaction by cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2003; **197:**129–35.
157 Darlington PJ, Baroja ML, Chau TA, Siu E, Ling V, Carreno BM, Madrenas J. Surface cytotoxic T lymphocyte-associated antigen 4 partitions within lipid rafts and relocates to the immunological synapse under conditions of inhibition of T cell activation. J Exp Med 2002; **195:**1337–47.
158 Kabouridis PS. Selective interaction of LAT (linker of activated T cells) with the open-active form of Lck in lipid rafts reveals a new mechanism for the regulation of Lck in T cells. Biochem J 2003; **371:**907–15.
159 van der Merwe PA, The TCR. Triggering puzzle. Immunity 2001; **14:**665–8.
160 Irles C, Symons A, Michel F, Bakker TR, van der Merwe PA, Acuto O. CD45 ectodomain controls interaction with GEMs and Lck activity for optimal TCR signaling. Nat Immunol 2003; **4:**189–97.
161 Edmonds SD, Ostergaard HL. Dynamic association of CD45 with detergent-insoluble microdomains in T lymphocytes. J Immunol 2002; **169:**5036–42.
162 Jury EC, Kabouridis PS, Flores-Borja F, Mageed RA, Isenberg DA. Altered lipid raft-associated signaling and ganglioside expression in T lymphocytes from patients with systemic lupus erythematosus. J Clin Invest 2004; **113:** 1176–87.
163 Krishnan S, Nambiar MP, Warke VG, Fisher CU, Mitchell J, Delaney N, Tsokos GC. Alterations in lipid raft composition

and dynamics contribute to abnormal T cell responses in systemic lupus erythematosus. J Immunol 2004; **172:**7821–31.
164 Jury EC, Kabouridis PS. T lymphocyte signalling in systemic lupus erythematosus: a lipid raft perspective. Lupus 2004; **13:**413–22.
165 He X, Woodford-Thomas TA, Johnson KG, Shah DD, Thomas ML. Targeting of CD45 protein tyrosine phosphatase activity to lipid microdomains on the T cell surface inhibits TCR signaling. Eur J Immunol 2002; **32:**2578–87.
166 Rodgers W, Rose JK. Exclusion of CD45 inhibits activity of p56lck associated with glycolipid-enriched membrane domains. J Cell Biol 1996; **135:**1515–23.
167 Kosugi A, Sakakura J, Yasuda K, Ogata M, Hamaoka T. Involvement of SHP-1 tyrosine phosphatase in TCR-mediated signaling pathways in lipid rafts. Immunity 2001; **14:**669–80.
168 Yasuda K, Nagafuku M, Shima T *et al*. Fyn is essential for tyrosine phosphorylation of Csk-binding protein/phosphoprotein associated with glycolipid-enriched microdomains in lipid rafts in resting T cells. J Immunol 2002; **169:**2813–7.
169 Torgersen KM, Vang T, Abrahamsen H *et al*. Release from tonic inhibition of T cell activation through transient displacement of C-terminal Src kinase (Csk) from lipid rafts. J Biol Chem 2001; **276:**29313–8.
170 Mustelin T, Tasken K. Positive and negative regulation of T-cell activation through kinases and phosphatases. Biochem J 2003; **371:**15–27.
171 Filipp D, Zhang J, Leung BL, Shaw A, Levin SD, Veillette A, Julius M. Regulation of Fyn through translocation of activated Lck into lipid rafts. J Exp Med 2003; **197:**1221–7.
172 Filipp D, Leung BL, Zhang J, Veillette A, Julius M. Enrichment of lck in lipid rafts regulates colocalized fyn activation and the initiation of proximal signals through TCR alpha beta. J Immunol 2004; **172:**4266–74.
173 Gomez-Mouton C, Abad JL, Mira E *et al*. Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. Proc Natl Acad Sci USA 2001; **98:**9642–7.
174 Seveau S, Eddy RJ, Maxfield FR, Pierini LM. Cytoskeleton-dependent membrane domain segregation during neutrophil polarization. Mol Biol Cell 2001; **12:**3550–62.
175 Pierini LM, Eddy RJ, Fuortes M, Seveau S, Casulo C, Maxfield FR. Membrane lipid organization is critical for human neutrophil polarization. J Biol Chem 2003; **278:**10831–41.
176 Manes S, Ana Lacalle R, Gomez-Mouton C, Martinez AC. From rafts to crafts: membrane asymmetry in moving cells. Trends Immunol 2003; **24:**320–6.