[Frontiers in Bioscience 11, 2929 -2939, September 1, 2006]

Negative regulation of T-cell receptor activation by the cAMP-PKA-Csk signalling pathway in T-cell lipid rafts

Kjetil Tasken and Anja Ruppelt

Biotechnology Centre of Oslo, University of Oslo, N-0317 Oslo, Norway

TABLE CONTENTS

1. ABSTRACT

Spatial organization of signal proteins in specialized cholesterol and glycosphingolipid-enriched microdomains (lipid rafts) provide specificity in lymphocyte signalling. Src kinases associate with lipid rafts on the basis of their dual acylation in the N-terminus and initiate T cell signalling. The immunomodulatory signal enzyme protein kinase A (PKA) is a serine/threonine kinase that controls a number of processes important for immune activation by phosphorylation of substrates that alters protein-protein interactions or changes the enzymatic activity of target proteins in T cells. PKA substrates involved in immune activation include transcription factors, members of the MAP kinase pathway, phospholipases and the Src kinase Csk. The PKA type I isoenzyme localizes to lipid rafts during T cell activation and modulates directly the proximal events that take place after engagement of the T cell receptor. The most proximal and major target for PKA phosphorylation is the C-terminal Src kinase Csk which initiates a negative signal pathway that fine-tunes the T cell activation process.

2. INTRODUCTION

Engagement of the T cell receptor/CD3 (TCR/CD3) complex can lead to a wide range of responses spanning from anergy and apoptosis to T cell activation with cytokine production, cytotoxic activity and proliferation. An optimal immune response requires the antigen to be presented to the T cell by an antigen presenting cell (the primary stimulus) in conjunction with a costimulatory stimulus (the secondary stimulus, e.g. CD28 or IL-2). Engagement of the TCR/CD3-complex elicits a signaling cascade in the T cell that involves numerous signaling molecules including protein tyrosine kinases (PTKs), protein tyrosine phosphatases (PTPs), G-proteins, GEFs and adaptor molecules (1,2). The first event is activation of the Src family PTKs Lck and Fyn, which subsequently leads to phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) present in the ζ, ε, δ and γ subunits of the TCR/CD3 (3,4). The phosphorylation of the ITAMs promotes recruitment and subsequent activation of the Syk-PTK ZAP-70. The activation of Src and Syk/ZAP-70 PTKs leads to

phosphorylation of adaptor molecules and recruitment of enzymes facilitating the activation of downstream signaling pathways (2). These events take place in specialized microdomains of the plasma membrane termed lipid rafts that have a high constituency of cholesterol and glycosphingolipids (5). The activation cascade culminates in gene transcription, cytoskeletal rearrangement, cytokine production and proliferation.

Activation of cAMP signaling involves binding of an extracellular ligand to a G-protein coupled receptor (GPCR) which through G-proteins regulates one of several isoforms of adenylyl cyclase leading to generation of cAMP. Although other effectors of cAMP have been identified (6,7), the most versatile effector system is PKA. Ligand-induced changes in cAMP concentration vary in duration, amplitude and localization in the cell. Spatiotemporal organization of cAMP microdomains are created through coupled control of cAMP production by adenylyl cyclases in conjunction with phosphodiesterases that degrade cAMP. Different PKA isozymes with distinct biochemical properties and cell-specific expression contribute to cell and organ specificity (for review and references, see (8,9)). A kinase anchoring proteins (AKAPs) target PKA to specific substrates and distinct subcellular compartments providing spatial and temporal specificity for mediation of biological effects induced through the cAMP-PKA pathway (for review and references, see (10-12)). AKAPs also serve as scaffolding proteins that assemble PKA together with signal proteins such as phosphatases and cAMP-specific phosphodiesterases as well as components of other signaling pathways into multiprotein signaling complexes that serve as crossroads for different signalling pathways. Targeting of PKA and integration of a wide repertoire of proteins involved in signal transduction into complex signal networks further increase the specificity required for the precise regulation of numerous cellular and physiologic processes. The cAMP-PKA pathway is strongly involved in the regulation and modulation of immune responses and is the most potent and acute inhibitor of activation of lymphocytes. Mechanisms of cAMP-mediated immunomodulation are discussed below.

3. LIPID RAFTS AS SIGNALING PLATFORMS

Cell membranes are mainly consisting of lipids assembled of polyunsaturated fatty acids that form a homogenous lipid double layer. In addition, the plasma membrane contains microdomains enriched in cholesterol and sphingolidids called lipid rafts or glycosphingolipid-enriched membane microdomain (GEMs). One specific physical feature of these regions is their resistance to standard detergents like Triton-X100 in the cold. Hydrophobic interactions between long saturated fatty acids and sphingolipids keep the microdomains insoluble and permit their separation from the residual plasma membrane and other cell content by density-gradient centrifugation.

Lipid rafts have a submicroscopic size of around 3-4 nm and are assembled to form macrostructures that are micrometers in diameter during the formation of an immunological synapse, see e.g. (12). This probably occurs due to rearrangement of the underlying actin cytoskeleton that anchors the lipid rafts. The connections of intermediate linker proteins between the membrane and the actin cytoskeleton are precisely regulated and provide an actin – raft cooperation. While most transmembrane proteins reside outside lipid rafts, some lipid-modified proteins associate specifically with lipid rafts. In particular, membrane proteins that take part in the proximal signaling events from the T cell receptor such as CD4, CD8, CD44, CD43 in addition to transmembrane adaptor molecules like LAT (linker for activation of T-cells), LIME (Lck interacting membrane protein) and Cbp/PAG (CSk binding protein/Protein Associated with Glycosphinglipid-enriched membrane microdomains), localize to lipid rafts. Proteins without a transmembrane domain, but that are modified by lipids such as double acylation, also accumulate in rafts including Src-family kinases, PIP2 (13) and small G-proteins. Analyses of lipid raft purifications from normal resting T cells for the presence of different subunits of PKA also reveal both the catalytic subunit and the regulatory subunit RI a (but no RII subunits) to be constitutively associated with rafts (14).

4. INITIATION OF THE T-CELL SIGNALING CASCADE

T cells contribute to an immune response by both direct effector functions and by modulating the function of other cell types by cell-cell contact and secretion of cytokines. The functional properties of T cells are partly segregated into different subsets such as CD4 T cells, CD8 T cells and regulatory T cells and are further refined by the differentiation status of the particular subset. However, one single T cell is able to mount various responses depending on the antigen, the mode of activation and the specific microenvironment. A proper T cell response is essential for the maintenance of normal immune homeostasis and for an effective immune protection. Conversely, abnormalities in their function can lead to autoimmunity, allergic manifestations or immunodeficiencies.

The primary event leading to the activation and differentiation of T cells is the triggering of their antigen-specific T cell receptor (TCR) by a processed antigenic peptide presented by a major histocompatibility complex (MHC) class II molecule on the surface of an antigen-presenting cell (APC) or by a MCH class I molecule on a target cell. This event triggers several signal transduction pathways that involve second messengers, protein kinases, protein phosphatases and other enzymes and key intermediates (15-18). The signaling cascade culminates with the induction of gene transcription according to defined genetic programs that are characteristic of the different T cell subsets, leading to the differentiation and T cell proliferation. In addition, the proper development and selection of immature T cells in the thymus also depends on similar signaling events that determine the selection of T cells with the appropriate antigen specificity and affinity.

It is generally agreed that a key initiating event in T cell activation by antigen is the increased

phosphorylation of ITAM tyrosines in TCR subunits by the Src family kinases Lck and Fyn (and perhaps c-Yes). Exactly how the TCR couples antigen recognition to this phosphorylation event is not clear. Proposed models include i) Juxtaposition of Lck to the TCR mediated by CD4 or CD8 (15,19-24), ii) Increased local concentration of kinases and their substrates following receptor oligomerization and lipid raft coalescence (25-31), and iii) The active exclusion of Csk and PTPases (32). These mechanisms are not mutually exclusive, and probably all operate in concert.

Phosphorylated ITAMs in CD3 ζ serves to recruit PTK Zap70 (ζ-chain–associated protein 70 kDa), which subsequently phosphorylate LAT (linker for activation of T-cells), SLP76 (SRC-homology 2 (SH2)-domain-containing leukocyte protein 76 kDa) and PLC γ1 (Phospholipase C γ1). The signaling cascade continues with the recruitment of (catalytic and non-catalytic) proteins such as Grb2 (Growth-factor–receptor-bound protein2), Gads (Grb2-related adaptor protein), the p85 subunit of PI3k, and NCK to LAT, that propagates intracellular signaling pathways that involves activation of RAS, PKC, mobilization of Ca^{2+}, calcineurin and polymerization of the actin cytoskeleton. A complete immune response finally leads to activation of transcription factor NFkB (nuclear factor KB) and NFAT (nuclear factor of activated T-cells) initiating cytokine secretion and T cell proliferation (33-36)

5. SUPPRESSION OF Src-FAMILY PTKs BY Csk

Since Lck and Fyn play a pivotal role in the first signal events from the T cell receptor, it is not surprising that they are tightly regulated by multiple mechanisms (reviewed in (31,33)). Similar mechanisms are also present in other cell types that express different members of the Src family PTKs. An important mode of regulation of Src-family PTKs is by phosphorylation at a conserved tyrosine residue within the catalytic domain, Y394 in Lck and Y417 in Fyn(T). It is generally believed that Src-family PTKs autophosphorylate at this site, presumably in trans, but there is also evidence that another PTK may be involved (37). Phosphorylation at this site in Lck relieves an intramolecular block of substrate binding and also provides a conformational change in the so called "activation loop" (where Y394 resides) to position the catalytic amino acid residues for optimal catalysis (38). Although the stoichiometry of Y394 phosphorylation in intact T cells is very low (39,40), this event is crucial for substrate phosphorylation by Lck and for transformation by oncogenic Lck (41,42). The low stoichiometry is mainly due to rapid dephosphorylation (43).

Both Lck and Fyn (as well as other Src family PTKs) are negatively regulated by phosphorylation at a conserved C-terminal regulatory tyrosine residue, Y505 in Lck and Y528 in Fyn(T) (44,44,45). When phosphorylated, the tail tyrosine binds to the Src homology 2 (SH2) domain of the same kinase molecule thereby forcing it into an inactive conformation (46). The crystallization of C-terminally phosphorylated Src (47) and Hck (48) also revealed that the SH3 domain is important for stabilizing the kinase domain in the inactive conformation by binding to a region in the linker between the SH2 domain and the kinase domain. At least in T cell lines, approximately half of all Lck molecules are phosphorylated at Y505 (49), but changes in the phosphorylation status of Y505 have not been observed during T cell activation. Instead, the phosphotyrosine content of Lck increases after T cell activation (50). This suggests that the activity of Lck in TCR signaling is not dependent on acute dephosphorylation at Y505.

The PTK responsible for the suppressive phosphorylation of Lck at Y505 and Fyn at Y528 is the C-terminal Src kinase (Csk), a widely expressed 50-kDa enzyme (51-54). At present, Csk is the only PTK known to have this specificity. The finding that disruption of the csk gene is lethal (55,56) and that cells recovered from the early embryos contain greatly activated Src family PTKs, suggests that no other gene can fully compensate for the loss of csk during embryogenesis. Furthermore, both inducible knock outs and mature T cells with acute elimination of Csk exibit increased Src family kinase activation and are hyperresponsive to T cell stimuli (57-59). Nevertheless, it is possible that the other Csk family member Csk homologous kinase (CHK) also functioning as negative regulatory kinases of the SFKs (reviewed in (60)), may participate in the suppression of Src family PTKs in other cell types.

Consistent with a negative role of Csk in the regulation of Src family PTKs, Chow and collaborators have demonstrated that overexpression of Csk in T cells leads to a marked reduction of TCR-induced tyrosine phosphorylation and IL-2 production. The activity of Csk required both the SH2 and SH3 domains and was further enhanced by targeting Csk to the plasma membrane (61). One explanation for the requirement of the SH3 domain is that that Csk specifically associates through its SH3 domain with the PEST-enriched PTPase (PEP) and it's human ortolog lymphoid protein tyrosine phosphatase (LYP)) (62). Thus, the targeting of Csk to the plasma membrane may not only increase its access to Src-related PTKs but also recruit PEP/LYP to the plasma membrane and improve its activity leading to dephosphorylation of membrane-associated proteins (*e.g.* Lck or Fyn).

In line with this, mice lacking PEP have exaggerated T cell expansion (63) and patients with a single-nucleotide polymorphism in the gene (PTPN22) encoding the LYP is associated with type 1 diabetes, rheumatoid arthritis, lupus, Graves thyroiditis, Addison disease and other autoimmune disorders (64-67). The disease associated-variant result in a R620W amino acid substitution in the SH3 binding sequence making LYP unable to bind Csk (64). Furthermore, in addition to the loss of Csk binding, the autoimmune-predisposing allele is a gain-of-function mutant where LYP-R620W has higher catalytic activity and is a more potent negative regulator of T lymphocyte activation (68).

6. SPATIOTEMPORAL REGULATION OF Csk RELEASES Lck FROM INHIBITION AND ALLOWS T CELL ACTIVATION TO OCCUR

Csk has a similar intramolecular organization to that of Src family PTKs with SH3-, SH2,-, and kinase

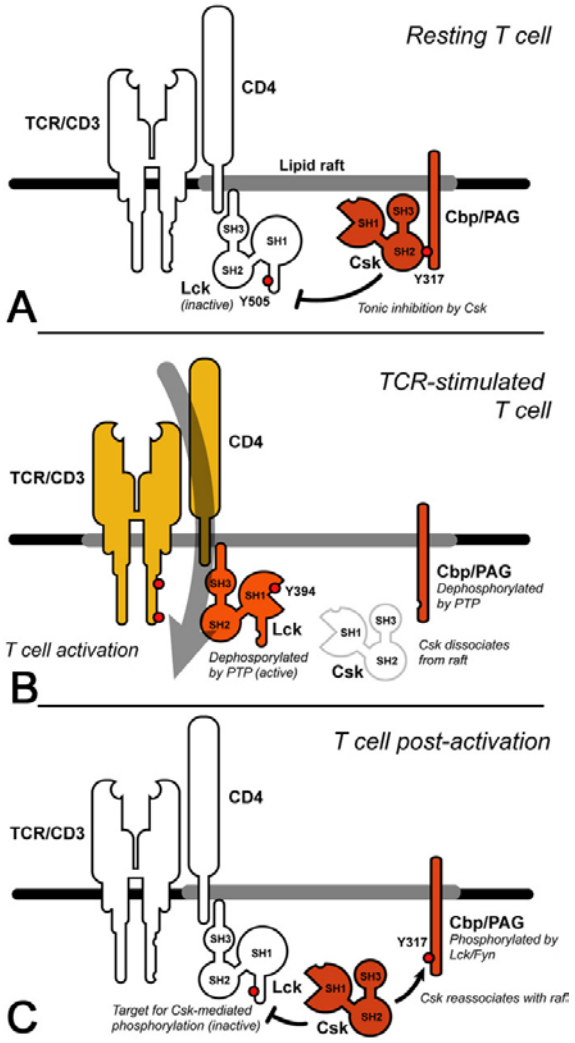

Figure 1. Csk is temporarily sent off duty during T cell activation. In resting normal T cells (a), Csk is present in lipid rafts through interaction with PY317 in Cbp/PAG and imposes a tonic inhibition of T cell activation through Csk-mediated phosphorylation of the Lck regulatory site (Y505). Engagement of the T cell receptor (b) results in CD45-dependent dephosphorylation of Cbp, dissociation of Csk from lipid rafts and displacement from its substrate Lck. This leads to activation of Lck and allowing for initiation of the TCR-induced tyrosine phosphorylation cascade. However, after 2-5 minutes of activation (c), Cbp/PAG Y317 is re-phosphorylated by Fyn thereby recruiting Csk back into lipid rafts. Together with Y394 dephosphorylation by PEP or SHP-1, this terminates Lck and Fyn activity and turns off TCR signaling.

domains, but differs from the Src kinases by the lack of the C-terminal regulatory tyrosine and autophosphorylation sites as well as the N-terminal lipid modification sequence that targets members of the Src kinase family to membranes. For that reason, it has for some time been unclear how this ubiquitously expressed cytosolic kinase effectively regulates the membrane associated Src kinases Lck and Fyn even though Csk has been shown to target to sites of Src activity in fibroblasts (69). The cloning and characterization of a transmembrane adapter protein, Csk binding protein (Cbp) or Phosphoprotein Associated with Glycosphingolipid enriched membrane domains (PAG), associated with lipid rafts has revealed new insight into the Csk-mediated regulation of Src kinases (70,71). Cbp/PAG binds to the SH2 domain of Csk and was originally observed as an 80-kDa phosphoprotein present in lipid rafts (72,73). It is ubiquitously expressed and likely interacts with actin filaments through the EBP50-ERM protein complex (74,75). Furthermore, Cbp/PAG has a palmitoylation site and a cytoplasmatic tail with 10 sites for tyrosine phosphorylation that is phosphorylated primarily by Fyn in T cells (76) whereas Cbp/PAG dephosphorylation is mediated by CD45 or PTPα (77,78). Csk, Fyn and EBP50 are reproducibly found to interact with Cbp/PAG *in vivo* and Tyr317 (Tyr314 in mouse and rat) is identified as the binding site for the SH2-domain of Csk, while Fyn seems to interact independently of the Cbp/PAG phosphotyrosine status (76). Interestingly, stimulation of T cells with antibodies towards CD3 alone or in combination with CD28 induces dephosphorylation of Cbp/PAG resulting in decreased Csk content in lipid rafts (32,70). We have further demonstrated that Csk association with Cbp/PAG is transient and corresponds with the kinetics of T cell activation (32). Overexpression of kinase-deficient Csk that displaces endogenous Csk from lipid rafts, have stimulatory effects on ζ-chain phosphorylation and IL-2 promoter activation both in resting T cells and after TCR triggering (32). Therefore, tonic suppression of Lck kinase activity in rafts by Csk seems to set the threshold for TCR signaling and appears important in order to avoid improper TCR signaling and T cell activation. Regulation of Csk kinase activity in rafts may therefore be crucial to prevent aberrant immune activation and autoimmunity. Although not completely understood, T cell activation requires transient dissociation of Csk from rafts, allowing Lck to become activated and to perform its catalytical functions, which provides an elegant mechanism for modulation of proximal TCR signaling (Figure 1). However, Cbp/PAG knock out studies indicate that Cbp/PAG is dispensable both for embryogenesis and T-cell development and function and suggests that Cbp/PAG may be redundant and that other Csk binding proteins may exist in rafts (79,80).

Concomitant with the activation of Lck and dissociation of Csk from lipid rafts, Csk associates with a 72-kDa protein via its SH2 domain (81). This protein was recently identified as G3BP, a phosphotyrosine-containing protein, reported to bind the SH3 domain of Ras GTPase-activating protein and seems to serve to sequester Csk outside lipid rafts (82). The formation of a complex of Csk and G3BP in T cells occurs within a time course that is similar to that of Csk-dissociation from Cbp/PAG upon TCR stimulation.

In addition, one major aspect in unraveling the processes controlling the initiation of TCR signaling would undoubtedly be the identification and characterization of the PTPases responsible for dephosphorylating Cbp/PAG. In addition to CD45 (83,84), the protein-tyrosine

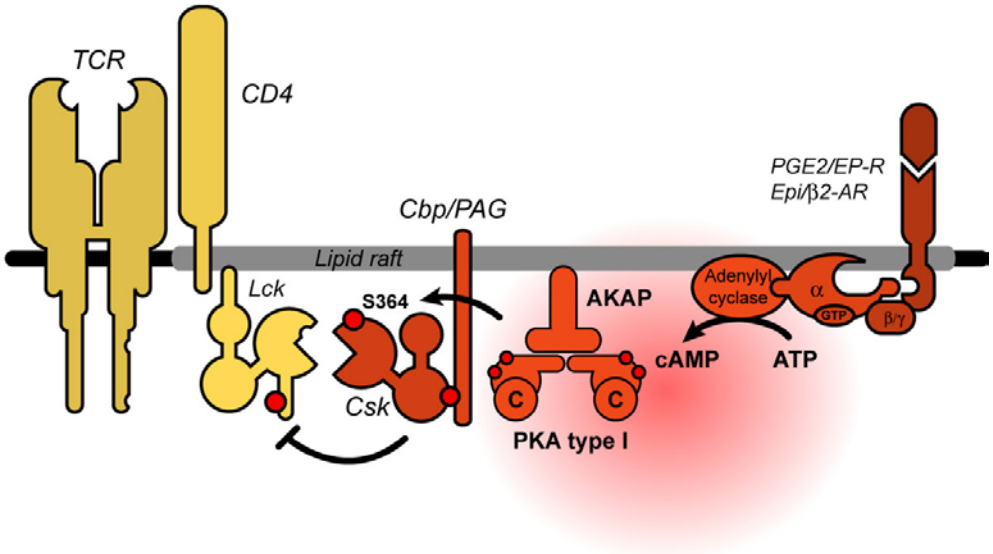

Figure 2. Cyclic AMP inhibits T cell activation through a PKA type I-Csk-Lck inhibitory pathway. In T cells, cAMP inhibits T cell receptor-induced T cell activation and thereby exerts important immunoregulatory functions (reviewed in (91) through a receptor – G-protein – adenylyl cyclase - cAMP - PKA type I – C-terminal Src kinase (Csk) inhibitory pathway assembled in T cell lipid rafts and acting on the Src family kinase Lck (14,88,95).

phosphatase Shp-2 appears to be involved in the phosphorylation status of the Csk–adaptor protein Cbp/PAG (85). C-terminal tyrosines are hyperphosphorylated in Shp-2 deficient cells which leads to decreased tyrosine phosphorylation in substrates of the Src family kinases. Shp regulates both the activation of Src family kinases and the activation of Ras/Erk signalling pathway.

7. REGULATION OF PROXIMAL T-CELL SIGNALING BY PKA TYPE I

Prostaglandin E_2 (PGE_2) and other ligands that lead to cAMP production by binding to G protein-coupled receptors inhibit TCR-induced T cell activation and thereby exert important immunoregulatory functions (86). Based on studies with selective agonists, activation of protein kinase A (PKA) type I ($RI\alpha_2C_2$) has been shown to be necessary and sufficient for mediating these effects of cAMP (87,88). Similarly, PKA type I negatively regulates activation of B cells through the B cell antigen receptor (89) and NK cell cytotoxicity elicited through specific NK cell receptors (90). Although PKA can modulate TCR signaling at multiple levels (reviewed in (91)), the observed inhibitory effects of cAMP on TCR-induced ζ-chain phosphorylation point towards an important role for Csk, which is the most up-stream PKA target reported so far. PKA phosphorylates S364 in Csk and induces a 2 to 4-fold increase in phosphotransferase activity of Csk in lipid rafts of T cells (14) (Figure 2).

Analyses of lipid raft purifications from normal resting T cells for the presence of different subunits of PKA, revealed that both the catalytic subunit and the regulatory subunit RIα (but no RII subunits) are constitutively associated with the lipid rafts (14). Similarly catalytic subunits Cα and Cβ2 associate with lipid rafts ((92) and our unpublished data). This suggests that the co-localization of PKA type I and TCR in capped T cells (88) occurs in lipid rafts and that there are mechanisms for specific targeting of PKA type I to these areas that involves interaction with an AKAP in lipid rafts (our unpublished results). However, other possibilities include anchoring of the PKA catalytic subunit via the N-terminal myristyl group into rafts, or via interactions with a caveolin-like protein in T cell rafts, similar to the PKA Cα interaction with caveolin in other cell types (93).

Studies of the organization of G proteins in the plasma membrane have revealed that in addition to G proteins, lipid rafts also contains adenylyl cyclase activity (94). In fact, a substantial fraction of the total isoproterenol or forskolin-stimulated adenylyl cyclase in S49 lymphoma cells is present in these fractions, strongly suggesting that the receptor-G protein and G protein-adenylyl cyclase coupling occur in lipid rafts, and similar data have been obtained for normal T cells and HEK293 cells (95). This implies that the entire molecular machinery required for generation of cAMP and activation of PKA type I after engagement of G-protein coupled receptors is recruited to lipid rafts and organized in a receptor-G-protein-AC-cAMP-PKA type I-Csk pathway scaffolded by an AKAP and Cbp/PAG (Figure 2).

8. REGULATION OF Csk ACTIVITY, A MOLECULAR MECHANISM FOR THE INHIBITORY EFFECT OF cAMP ON IMMUNE FUNCTIONS

So far, two different mechanisms are reported to regulate Csk activity. PKA, through phosphorylation of

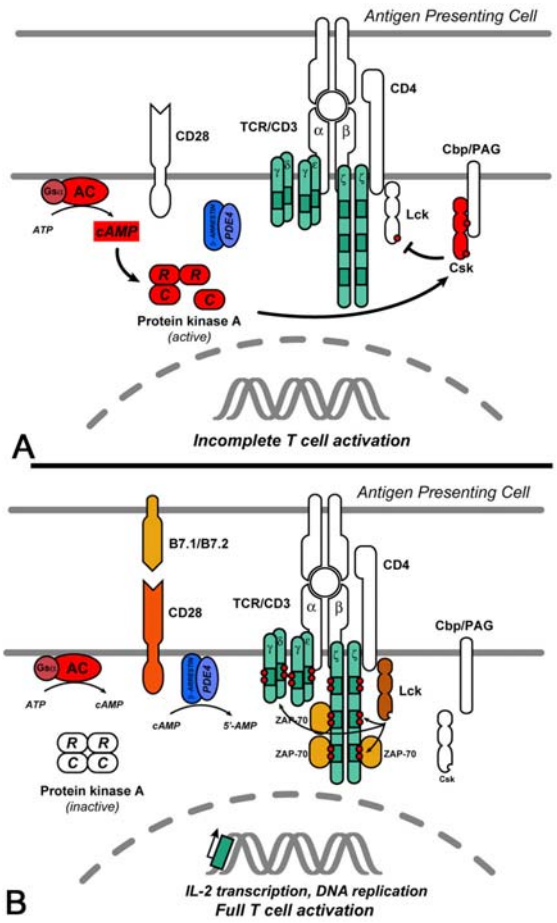

Figure 3. PKA and PDE4 have opposing functions in proximal T cell signalling. We propose a model where full T cell activation is prevented upon TCR triggering alone due to G protein coupling to the adenyl cyclase (AC) and generation of cAMP. Increased cAMP levels lead to activation of PKA, phosphorylation and activation of Csk and inhibition of Lck (a). However, CD28 co-stimulation down-modulates the TCR-induced cAMP-mediated inhibitory signals through recruitment of a β-arrestin/PDE4 complex leading to cAMP degradation and thus allowing a full T cell response to occur (b).

Ser-364 increases Csk kinase activity 2 to 4-fold leading to reduced Lck activity and ζ-chain phosphorylation. The other mechanism involves the adaptor molecule Cbp/PAG. Cbp/PAG recruits Csk to the site of action in lipid rafts (70,71), and the interaction between Csk-SH2 and Cbp/PAG through phosphorylated Y314/Y317 (rat/human Cbp/PAG) increases Csk activity (96). Addition of either recombinant Cbp/PAG or peptides corresponding to the Csk-SH2 binding site significantly increased Csk kinase activity towards a Src substrate *in vitro*. Thus, PKA phosphorylation of Csk and interaction with Cbp/PAG may act together in turning on Csk activity, providing a powerful mechanism for terminating activation through antigen receptors dependent on Src kinase signaling (Figure 2).

Interestingly, the cAMP inhibitory pathway has also been shown to be affected in several disease conditions. T cells from HIV infected patients have elevated levels of cAMP and hyperactivation of PKA (97). Targeting of the cAMP-PKA type I pathway by selective antagonists improves the T cell function *ex vivo* (97,98) and targeting cyclooxygenase 2 to reduce PGE_2 production lowers cAMP and increases T cell function *in vivo* ((99,100)). A similar mechanism contributes to the T cell dysfunction in a subset of patients with common variable immunodeficiency (CVI) (101), and to the severe T cell anergy in a murine immunodeficiency model termed MAIDS (mouse AIDS) (102).

9. UNCOUPLING FROM cAMP IMMUNOMODULATION BY RECRUITMENT OF A PHOSPHODIESTERASE THAT DEGRADES cAMP

On the basis of the inhibitory role of cAMP, one might expect the intracellular level of cAMP to fall upon T cell activation. However, more than 15 years ago, the intracellular cAMP levels were reported to increase following T cell activation in the presence of the PDE inhibitor IBMX (86,103). Both the significance of activation-induced cAMP production and the intracellular location where cAMP production takes place, has been ill-understood to date. However, we have recently shown that T cell activation leads to rapid production of cAMP in lipid rafts in a G-protein dependent fashion, resulting in raft-associated PKA activation (104) (Figure 3A).

The spatiotemporal regulation of intracellular cAMP gradients is a result of the combined action of adenylyl cyclases and PDEs, which provide the sole route of cAMP degradation (105-107). The importance of PDE action in T cells is clearly evident from both previous studies by other investigators (108-111) and also from our experiments showing that PDE inhibition augments the increase in cAMP caused by T cell activation (112). Not only are PDE4 enzymes the main contributors of cAMP-PDE activity in T cells (108) but PDE4 selective inhibitors exert a major component of their anti-inflammatory action through attenuation of T cell function (113,114). Thus one might expect that PDE4 activity would serve to promote T cell activity by reducing cAMP levels in the appropriate intracellular compartment. It is therefore interesting that TCR stimulation lead to increased adenylyl cyclase activity and cAMP production (87,115). However, we have recently shown that cAMP production elicited by TCR engagement is ablated upon CD28 co-stimulation. CD28 co-stimulation recruits PDE4 to the lipid raft fraction. Recruitment of PDE4 to rafts is accompanied by recruitment of β-arrestin, which in various other cell types has been shown to play an important role in uncoupling the receptor-mediated stimulation of G_s (116) and allowing the receptor-mediated recruited of PDE4 isoforms (117,118).

In addition to recruitment of PDE4 isoforms to lipid rafts upon T-cell stimulation, it is possible that phosphorylation of PDE4 could further contribute to the increased phosphodiesterase activity. The activity of the long PDE4A4 isoform has been shown to be improved by

about 25% through phosphorylation by PKA (119,120) and the activity of the short PDE4B2 and PDE4D1 isoforms can be similarly improved through the action of ERK (121), which becomes activated upon TCR stimulation (122). However, the paucity of PDE4 protein in rafts makes a direct demonstration of this using either direct phosphorylation or the use of phospho-antibodies difficult. There are also other PDEs found in T cells (109,123), but no change in the activity of PDE3 was detected in lipid rafts upon TCR co-stimulation. PDE7 has been suggested to be important for T cell proliferation as it is up-regulated during the first eight hours of T cell activation. However, it is absent from resting T cells (123) and T cell functioning has recently been shown to be normal in knockout mice (124) indicating that PDE7 is unlikely to be involved in the regulation of the initial T cell signaling events.

Stimulation of the TCR is known to induce a signal that is too weak to fully activate T cells (125). The signal can, however, be amplified by CD28 co-stimulation and together these two signals is able to induce full T cell activation and clonal expansion (126). It is therefore interesting that CD28-induced recruitment of β-arrestin and PDE4 appears to play a key role in constraining TCR-induced adenylyl cyclase activation by recruitment of PDE4 in complex with β-arrestin (104) (Figure 3B). The recruited β-arrestin may also serve an uncoupling role, although the demonstration of this and the identification of its partner allowing recruitment will be a challenge for future studies. Since anti-CD3 and anti-CD28 co-stimulation recruits and activates PDE4 to a greater extent than anti-CD3 stimulation alone, signal-amplification by co-stimulation may be mediated through activation of unknown molecules in addition to identified species such as PI3-kinase, Itk and Vav-1 (125). We suggest an additional role for CD28 as a molecular amplifier of TCR-induced signals whereby CD28 mediates PDE4 recruitment to lipid rafts and thereby suppress the inhibitory action of cAMP signals (Figure 3).

10. SUMMARY AND PERSPECTIVES

The activities of both PKA and PDE4 seem to be important for regulation of TCR-induced signalling and T cell function. PKA is activated by external stimuli such as PGE_2 and adrenergic agonists as well as by an intrinsic mechanism upon TCR stimulation both of which induces cAMP production in lipid rafts and inhibits proximal T cell signaling (Figure 3A). However, overexpression of PDE4 isoforms or β-arrestin has been demonstrated to increase T cell activation revealing regulatory roles for both proteins in T cell signaling, and a PDE4/β-arrestin complex is recruited to lipid rafts in response to a CD28 co-signal. We propose a novel role for TCR and CD28 co-stimulation in down-modulation of TCR-induced cAMP-mediated inhibitory signals through the recruitment of β-arrestin and PDE4 to lipid rafts and thus allowing a full T cell response to occur (Figure 3B).

10. ACKNOWLEDGMENTS

We are grateful to Dr. Einar Martin Aandahl for critical reading of the manuscript and preparation of illustrations. Our work is supported by grants from the Functional Genomics Programme, The Research Council of Norway and The Norwegian Cancer Society.

11. REFERENCES

1. Mustelin T. & K. Tasken: Positive and negative regulation of T cell activation through kinases and phosphatases. *Biochem J* 371, 15-27 (2003)
2. Leo A. & B. Schraven: Adapters in lymphocyte signalling. *Curr Opin Immunol* 13, 307-316 (2001)
3. Latour S. & A. Veillette: Proximal protein tyrosine kinases in immunoreceptor signaling. *Curr Opin Immunol* 13, 299-306 (2001)
4. Billadeau D. D. & P. J. Leibson: ITAMs versus ITIMs: striking a balance during cell regulation. *J Clin Invest* 109, 161-168 (2002)
5. Xavier R., T. Brennan, Q. Li, C. McCormack & B. Seed: Membrane compartmentation is required for efficient T cell activation. *Immunity* 8, 723-732 (1998)
6. de Rooij J., F. J. T. Zwartkruis, M. H. G. Verheijen, R. H. Cool, S. M. B. Nijman, A. Wittinghofer & J. L. Bos: Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396, 474-477 (1998)
7. Kaupp U. B. & R. Seifert: Cyclic nucleotide-gated ion channels. *Physiol Rev* 82, 769-824 (2002)
8. Skalhegg B. S. & K. Tasken: Specificity in the cAMP/PKA signaling pathway. Differential expression, regulation, and subcellular localization of subunits of PKA. *Front Biosci* 5, D678-D693 (2000)
9. Tasken K., B. S. Skalhegg, K. A. Tasken, R. Solberg, H. K. Knutsen, F. O. Levy, M. Sandberg, S. Orstavik, T. Larsen, A. K. Johansen, T. Vang, H. P. Schrader, N. T. K. Reinton, K. M. Torgersen, V. Hansson & T. Jahnsen: Structure, function, and regulation of human cAMP-dependent protein kinases. *Signal Transduction in Health and Disease* 31, 191-204 (1997)
10. Michel J. J. & J. D. Scott: AKAP mediated signal transduction. *Annu Rev Pharmacol Toxicol* 42, 235-257 (2002)
11. Diviani D. & J. D. Scott: AKAP signaling complexes at the cytoskeleton. *J Cell Sci* 114, 1431-1437 (2001)
12. Dustin M. L.: Coordination of T cell activation and migration through formation of the immunological synapse. *Ann N Y Acad Sci* 987, 51-59 (2003)
13. Hope H. R. & L. J. Pike: Phosphoinositides and phosphoinositide-utilizing enzymes in detergent-insoluble lipid domains. *Mol Biol Cell* 7, 843-851 (1996)
14. Vang T., K. M. Torgersen, V. Sundvold, M. Saxena, F. O. Levy, B. S. Skalhegg, V. Hansson, T. Mustelin & K. Tasken: Activation of the COOH-terminal Src kinase (Csk) by cAMP-dependent protein kinase inhibits signaling through the T cell receptor. *J Exp Med* 193, 497-507 (2001)
15. Mustelin T., K. M. Coggeshall, N. Isakov & A. Altman: T cell antigen receptor-mediated activation of phospholipase C requires tyrosine phosphorylation. *Science* 247, 1584-1587 (1990)
16. June C. H., M. C. Fletcher, J. A. Ledbetter, G. L. Schieven, J. N. Siegel, A. F. Phillips & L. E. Samelson: Inhibition of tyrosine phosphorylation prevents T-cell receptor-mediated signal transduction. *Proc Natl Acad Sci U S A* 87, 7722-7726 (1990)

17. Altman A., K. M. Coggeshall & T. Mustelin: Molecular events mediating T cell activation. *Adv Immunol* 48, 227-360 (1990)
18. Klausner R. D. & L. E. Samelson: T-Cell Antigen Receptor Activation Pathways - the Tyrosine Kinase Connection. *Cell* 64, 875-878 (1991)
19. Mustelin T. & A. Altman: Do Cd4 and Cd8 Control T-Cell Activation Via A Specific Tyrosine Protein-Kinase. *Immunol Today* 10, 189-192 (1989)
20. Rudd C. E., J. M. Trevillyan, J. D. Dasgupta, L. L. Wong & S. F. Schlossman: The CD4 receptor is complexed in detergent lysates to a protein-tyrosine kinase (pp58) from human T lymphocytes. *Proc Natl Acad Sci U S A* 85, 5190-5194 (1988)
21. Veillette A., M. A. Bookman, E. M. Horak & J. B. Bolen: The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck. *Cell* 55, 301-308 (1988)
22. Barber E. K., J. D. Dasgupta, S. F. Schlossman, J. M. Trevillyan & C. E. Rudd: The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56lck) that phosphorylates the CD3 complex. *Proc Natl Acad Sci U S A* 86, 3277-3281 (1989)
23. Haughn L., S. Gratton, L. Caron, R. P. Sekaly, A. Veillette & M. Julius: Association of tyrosine kinase p56lck with CD4 inhibits the induction of growth through the alpha beta T-cell receptor. *Nature* 358, 328-331 (1992)
24. Emmrich F.: Cross-linking of CD4 and CD8 with the T-cell receptor complex: quaternary complex formation and T-cell repertoire selection. *Immunol Today* 9, 296-300 (1988)
25. Rodgers W., B. Crise & J. K. Rose: Signals determining protein tyrosine kinase and glycosyl-phosphatidylinositol-anchored protein targeting to a glycolipid-enriched membrane fraction. *Mol Cell Biol* 14, 5384-5391 (1994)
26. Janes P. W., S. C. Ley, A. I. Magee & P. S. Kabouridis: The role of lipid rafts in T cell antigen receptor (TCR) signalling. *Semin Immunol* 12, 23-34 (2000)
27. Kabouridis P. S., J. Janzen, A. L. Magee & S. C. Ley: Cholesterol depletion disrupts lipid rafts and modulates the activity of multiple signaling pathways in T lymphocytes. *Eur J Immunol* 30, 954-963 (2000)
28. Ishiai M., M. Kurosaki, K. Inabe, A. C. Chan, K. Sugamura & T. Kurosaki: Involvement of LAT, Gads, and Grb2 in compartmentation of SLP-76 to the plasma membrane. *J Exp Med* 192, 847-856 (2000)
29. Boerth N. J., J. J. Sadler, D. E. Bauer, J. L. Clements, S. M. Gheith & G. A. Koretzky: 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* 192, 1047-1058 (2000)
30. Viola A.: The amplification of TCR signaling by dynamic membrane microdomains. *Trends Immunol* 22, 322-327 (2001)
31. Mustelin T.: T cell antigen receptor signaling: three families of tyrosine kinases and a phosphatase. *Immunity* 1, 351-356 (1994)
32. Torgersen K. M., T. Vang, H. Abrahamsen, S. Yaqub, V. Horejsi, B. Schraven, B. Rolstad, T. Mustelin & K. Tasken: Release from tonic inhibition of T cell activation through transient displacement of C-terminal Src kinase (Csk) from lipid rafts. *J Biol Chem* 276, 29313-29318 (2001)
33. Myung P. S., N. J. Boerthe & G. A. Koretzky: Adapter proteins in lymphocyte antigen-receptor signaling. *Current Opinion in Immunology* 12, 256-266 (2000)
34. Rudd C. E.: Adaptors and molecular scaffolds in immune cell signaling. *Cell* 96, 5-8 (1999)
35. Kuo C. T. & J. M. Leiden: Transcriptional regulation of T lymphocyte development and function. *Annu Rev Immunol* 17, 149-187 (1999)
36. Serfling E., F. Berberich-Siebelt, S. Chuvpilo, E. Jankevics, S. Klein-Hessling, T. Twardzik & A. Avots: The role of NF-AT transcription factors in T cell activation and differentiation. *Biochim Biophys Acta* 1498, 1-18 (2000)
37. Hardwick J. S. & B. M. Sefton: Activation of the Lck tyrosine protein kinase by hydrogen peroxide requires the phosphorylation of Tyr-394. *Proc Natl Acad Sci U S A* 92, 4527-4531 (1995)
38. Yamaguchi H. & W. A. Hendrickson: Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation. *Nature* 384, 484-489 (1996)
39. Hurley T. R. & B. M. Sefton: Analysis of the activity and phosphorylation of the lck protein in lymphoid cells. *Oncogene* 4, 265-272 (1989)
40. Marth J. D., D. B. Lewis, C. B. Wilson, M. E. Gearn, E. G. Krebs & R. M. Perlmutter: Regulation of pp56lck during T-cell activation: functional implications for the src-like protein tyrosine kinases. *EMBO J* 6, 2727-2734 (1987)
41. Abraham N. & A. Veillette: Activation of p56lck through mutation of a regulatory carboxy-terminal tyrosine residue requires intact sites of autophosphorylation and myristylation. *Mol Cell Biol* 10, 5197-5206 (1990)
42. Veillette A. & M. Fournel: The CD4 associated tyrosine protein kinase p56lck is positively regulated through its site of autophosphorylation. *Oncogene* 5, 1455-1462 (1990)
43. Oetken C., M. von Willebrand, A. Marie-Cardine, T. Pessa-Morikawa, A. Stahls, S. Fisher & T. Mustelin: Induction of hyperphosphorylation and activation of the p56lck protein tyrosine kinase by phenylarsine oxide, a phosphotyrosine phosphatase inhibitor. *Mol Immunol* 31, 1295-1302 (1994)
44. Amrein K. E. & B. M. Sefton: Mutation of a site of tyrosine phosphorylation in the lymphocyte-specific tyrosine protein kinase, p56lck, reveals its oncogenic potential in fibroblasts. *Proc Natl Acad Sci U S A* 85, 4247-4251 (1988)
45. Kawakami T., Y. Kawakami, S. A. Aaronson & K. C. Robbins: Acquisition of transforming properties by FYN, a normal SRC-related human gene. *Proc Natl Acad Sci U S A* 85, 3870-3874 (1988)
46. Liu X., S. R. Brodeur, G. Gish, Z. Songyang, L. C. Cantley, A. P. Laudano & T. Pawson: Regulation of c-Src tyrosine kinase activity by the Src SH2 domain. *Oncogene* 8, 1119-1126 (1993)
47. Xu W., S. C. Harrison & M. J. Eck: Three-dimensional structure of the tyrosine kinase c-Src. *Nature* 385, 595-602 (1997)
48. Sicheri F., I. Moarefi & J. Kuriyan: Crystal structure of the Src family tyrosine kinase Hck. *Nature* 385, 602-609 (1997)
49. Sefton B. M.: The lck tyrosine protein kinase. *Oncogene* 6, 683-686 (1991)

50. Weber J. R., G. M. Bell, M. Y. Han, T. Pawson & J. B. Imboden: Association of the tyrosine kinase LCK with phospholipase C-gamma 1 after stimulation of the T cell antigen receptor. *J Exp Med* 176, 373-379 (1992)
51. Nada S., M. Okada, A. MacAuley, J. A. Cooper & H. Nakagawa: Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60c-src. *Nature* 351, 69-72 (1991)
52. Okada M., S. Nada, Y. Yamanashi, T. Yamamoto & H. Nakagawa: CSK: a protein-tyrosine kinase involved in regulation of src family kinases. *J Biol Chem* 266, 24249-24252 (1991)
53. Partanen J., E. Armstrong, M. Bergman, T. P. Makela, H. Hirvonen, K. Huebner & K. Alitalo: cyl encodes a putative cytoplasmic tyrosine kinase lacking the conserved tyrosine autophosphorylation site (Y416src). *Oncogene* 6, 2013-2018 (1991)
54. Bergman M., T. Mustelin, C. Oetken, J. Partanen, N. A. Flint, K. E. Amrein, M. Autero, P. Burn & K. Alitalo: The human p50csk tyrosine kinase phosphorylates p56lck at Tyr-505 and down regulates its catalytic activity. *EMBO J* 11, 2919-2924 (1992)
55. Imamoto A. & P. Soriano: Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice. *Cell* 73, 1117-1124 (1993)
56. Nada S., T. Yagi, H. Takeda, T. Tokunaga, H. Nakagawa, Y. Ikawa, M. Okada & S. Aizawa: Constitutive activation of Src family kinases in mouse embryos that lack Csk. *Cell* 73, 1125-1135 (1993)
57. Schmedt C., K. Saijo, T. Niidome, R. Kuhn, S. Aizawa & A. Tarakhovsky: Csk controls antigen receptor-mediated development and selection of T-lineage cells. *Nature* 394, 901-904 (1998)
58. Schmedt C. & A. Tarakhovsky: Autonomous maturation of alpha/beta T lineage cells in the absence of COOH-terminal Src kinase (Csk). *J Exp Med* 193, 815-826 (2001)
59. Vang T., H. Abrahamsen, S. Myklebust, J. Enserink, H. Prydz, T. Mustelin, M. Amarzguioui & K. Tasken: Knockdown of C-terminal Src kinase by siRNA-mediated RNA interference augments T cell receptor signaling in mature T cells. *Eur J Immunol* 34, 2191-2199 (2004)
60. Chong Y. P., T. D. Mulhern & H. C. Cheng: C-terminal Src kinase (CSK) and CSK-homologous kinase (CHK)--endogenous negative regulators of Src-family protein kinases. *Growth Factors* 23, 233-244 (2005)
61. Chow L. M., M. Fournel, D. Davidson & A. Veillette: Negative regulation of T-cell receptor signalling by tyrosine protein kinase p50csk. *Nature* 365, 156-160 (1993)
62. Cloutier J. F. & A. Veillette: Association of inhibitory tyrosine protein kinase p50csk with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. *EMBO J* 15, 4909-4918 (1996)
63. Hasegawa K., F. Martin, G. Huang, D. Tumas, L. Diehl & A. C. Chan: PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 303, 685-689 (2004)
64. Bottini N., L. Musumeci, A. Alonso, S. Rahmouni, K. Nika, M. Rostamkhani, J. MacMurray, G. F. Meloni, P. Lucarelli, M. Pellecchia, G. S. Eisenbarth, D. Comings & T. Mustelin: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 36, 337-338 (2004)
65. Begovich A. B., V. E. Carlton, L. A. Honigberg, S. J. Schrodi, A. P. Chokkalingam, H. C. Alexander, K. G. Ardlie, Q. Huang, A. M. Smith, J. M. Spoerke, M. T. Conn, M. Chang, S. Y. Chang, R. K. Saiki, J. J. Catanese, D. U. Leong, V. E. Garcia, L. B. McAllister, D. A. Jeffery, A. T. Lee, F. Batliwalla, E. Remmers, L. A. Criswell, M. F. Seldin, D. L. Kastner, C. I. Amos, J. J. Sninsky & P. K. Gregersen: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75, 330-337 (2004)
66. Siminovitch K. A.: PTPN22 and autoimmune disease. *Nat Genet* 36, 1248-1249 (2004)
67. Viken M. K., S. S. Amundsen, T. K. Kvien, K. M. Boberg, I. M. Gilboe, V. Lilleby, L. M. Sollid, O. T. Forre, E. Thorsby, A. Smerdel & B. A. Lie: Association analysis of the 1858C>T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. *Genes Immun* 6, 271-273 (2005)
68. Vang T., M. Congia, M. D. Macis, L. Musumeci, V. Orru, P. Zavattari, K. Nika, L. Tautz, K. Tasken, F. Cucca, T. Mustelin & N. Bottini: Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 37, 1317-1319 (2005)
69. Howell B. W. & J. A. Cooper: Csk suppression of Src involves movement of Csk to sites of Src activity. *Mol Cell Biol* 14, 5402-5411 (1994)
70. Brdicka T., D. Pavilstova, A. Leo, E. Bruyns, V. Korinek, P. Angelisova, J. Scherer, A. Shevchenko, A. Shevchenko, I. Hilgert, J. Cerny, K. Drbal, Y. Kuramitsu, B. Kornacker, V. Horejsi & B. Schraven: 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* 191, 1591-1604 (2000)
71. Kawabuchi M., Y. Satomi, T. Takao, Y. Shimonishi, S. Nada, K. Nagai, A. Tarakhovsky & M. Okada: Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. *Nature* 404, 999-1003 (2000)
72. Cinek T. & V. Horejsi: The nature of large noncovalent complexes containing glycosyl-phosphatidylinositol-anchored membrane glycoproteins and protein tyrosine kinases. *J Immunol* 149, 2262-2270 (1992)
73. Garnett D., A. N. Barclay, A. M. Carmo & A. D. Beyers: The association of the protein tyrosine kinases p56lck and p60fyn with the glycosyl phosphatidylinositol-anchored proteins Thy-1 and CD48 in rat thymocytes is dependent on the state of cellular activation. *Eur J Immunol* 23, 2540-2544 (1993)
74. Brdickova N., T. Brdicka, L. Andera, J. Spicka, P. Angelisova, S. L. Milgram & V. Horejsi: Interaction between two adapter proteins, PAG and EBP50: a possible link between membrane rafts and actin cytoskeleton. *FEBS Lett* 507, 133-136 (2001)
75. Itoh K., M. Sakakibara, S. Yamasaki, A. Takeuchi, H. Arase, M. Miyazaki, N. Nakajima, M. Okada & T. Saito: Cutting edge: Negative regulation of immune synapse

formation by anchoring lipid raft to cytoskeleton through Cbp-EBP50-ERM assembly. *J Immunol* 168, 541-544 (2002)
76. Yasuda K., M. Nagafuku, T. Shima, M. Okada, T. Yagi, T. Yamada, Y. Minaki, A. Kato, S. Tani-Ichi, T. Hamaoka & A. Kosugi: Cutting edge: 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* 169, 2813-2817 (2002)
77. Davidson D., M. Bakinowski, M. L. Thomas, V. Horejsi & A. Veillette: Phosphorylation-dependent regulation of T-cell activation by PAG/Cbp, a lipid raft-associated transmembrane adaptor. *Mol Cell Biol* 23, 2017-2028 (2003)
78. Maksumova L., H. T. Le, F. Muratkhodjaev, D. Davidson, A. Veillette & C. J. Pallen: Protein tyrosine phosphatase alpha regulates Fyn activity and Cbp/PAG phosphorylation in thymocyte lipid rafts. *J Immunol* 175, 7947-7956 (2005)
79. Dobenecker M. W., C. Schmedt, M. Okada & A. Tarakhovsky: The ubiquitously expressed Csk adaptor protein Cbp is dispensable for embryogenesis and T-cell development and function. *Mol Cell Biol* 25, 10533-10542 (2005)
80. Xu S., J. Huo, J. E. Tan & K. P. Lam: Cbp deficiency alters Csk localization in lipid rafts but does not affect T-cell development. *Mol Cell Biol* 25, 8486-8495 (2005)
81. Oetken C., C. Couture, M. Bergman, N. Bonnefoyberard, S. Williams, K. Alitalo, P. Burn & T. Mustelin: Tcr/Cd3-Triggering Causes Increased Activity of the P50(Csk) Tyrosine Kinase and Engagement of Its Sh2 Domain. *Oncogene* 9, 1625-1631 (1994)
82. Rahmouni S., T. Vang, A. Alonso, S. Williams, M. van Stipdonk, C. Soncini, M. Moutschen, S. P. Schoenberger & T. Mustelin: Removal of C-terminal Src kinase from the immune synapse by a new binding protein. *Mol Cell Biol* 25, 2227-2241 (2005)
83. Palacios E. H. & A. Weiss: Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. *Oncogene* 23, 7990-8000 (2004)
84. Mustelin T., A. Alonso, N. Bottini, H. Huynh, S. Rahmouni, K. Nika, C. Louis-dit-Sully, L. Tautz, S. H. Togo, S. Bruckner, A. V. Mena-Duran & A. M. al Khouri: Protein tyrosine phosphatases in T cell physiology. *Mol Immunol* 41, 687-700 (2004)
85. Zhang S. Q., W. T. Yang, M. I. Kontaridis, T. G. Bivona, G. Y. Wen, T. Araki, J. C. Luo, J. A. Thompson, B. L. Schraven, M. R. Philips & B. G. Neel: Shp2 regulates Src family kinase activity and Ras/Erk activation by controlling Csk recruitment. *Mol Cell* 13, 341-355 (2004)
86. Kammer G. M.: The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. *Immunol Today* 9, 222-229 (1988)
87. Skalhegg B. S., B. F. Landmark, S. O. Doskeland, V. Hansson, T. Lea & T. Jahnsen: Cyclic AMP-dependent protein kinase type I mediates the inhibitory effects of 3',5'-cyclic adenosine monophosphate on cell replication in human T lymphocytes. *J Biol Chem* 267, 15707-15714 (1992)
88. Skalhegg B. S., K. Tasken, V. Hansson, H. S. Huitfeldt, T. Jahnsen & T. Lea: Location of cAMP-dependent protein kinase type I with the TCR-CD3 complex. *Science* 263, 84-87 (1994)
89. Levy F. O., A. M. Rasmussen, K. Tasken, B. S. Skalhegg, H. S. Huitfeldt, S. Funderud, E. B. Smeland & V. Hansson: Cyclic AMP-dependent protein kinase (cAK) in human B cells: co-localization of type I cAK (RI alpha 2 C2) with the antigen receptor during anti-immunoglobulin-induced B cell activation. *Eur J Immunol* 26, 1290-1296 (1996)
90. Torgersen K. M., J. T. Vaage, F. O. Levy, V. Hansson, B. Rolstad & K. Tasken: Selective activation of cAMP-dependent protein kinase type I inhibits rat natural killer cell cytotoxicity. *J Biol Chem* 272, 5495-5500 (1997)
91. Torgersen K. M., T. Vang, H. Abrahamsen, S. Yaqub & K. Tasken: Molecular mechanisms for protein kinase A-mediated modulation of immune function. *Cell Signal* 14, 1-9 (2002)
92. Orstavik S., A. Funderud, T. T. Hafte, S. Eikvar, T. Jahnsen & B. S. Skalhegg: Identification and characterization of novel PKA holoenzymes in human T lymphocytes. *FEBS J* 272, 1559-1567 (2005)
93. Razani B., C. S. Rubin & M. P. Lisanti: Regulation of cAMP-mediated signal transduction via interaction of caveolins with the catalytic subunit of protein kinase A. *J Biol Chem* 274, 26353-26360 (1999)
94. Huang C., J. R. Hepler, L. T. Chen, A. G. Gilman, R. G. Anderson & S. M. Mumby: Organization of G proteins and adenylyl cyclase at the plasma membrane. *Mol Biol Cell* 8, 2365-2378 (1997)
95. Abrahamsen H., T. Vang & K. Tasken: Protein kinase A intersects Src signaling in membrane microdomains. *J Biol Chem* (2003)
96. Takeuchi S., Y. Takayama, A. Ogawa, K. Tamura & M. Okada: Transmembrane phosphoprotein Cbp positively regulates the activity of the carboxyl-terminal Src kinase, Csk. *J Biol Chem* 275, 29183-29186 (2000)
97. Aandahl E. M., P. Aukrust, B. S. Skalhegg, F. Muller, S. S. Froland, V. Hansson & K. Tasken: Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients. *FASEB J* 12, 855-862 (1998)
98. Aandahl E. M., P. Aukrust, F. Muller, V. Hansson, K. Tasken & S. S. Froland: Additive effects of IL-2 and protein kinase A type I antagonist on function of T cells from HIV-infected patients on HAART. *AIDS* 13, F109-F114 (1999)
99. Johansson C. C., T. Bryn, E. M. Aandahl, M. A. Areklett, P. Aukrust, K. Tasken & S. S. Froland: Treatment with type-2 selective and non-selective cyclooxygenase inhibitors improves T-cell proliferation in HIV-infected patients on highly active antiretroviral therapy. *AIDS* 18, 951-952 (2004)
100. Kvale D., V. Ormaasen, A. M. Kran, C. C. Johansson, P. Aukrust, E. M. Aandahl, S. S. Froland & K. Tasken: Immune modulatory effects of cyclooxygenase type 2 inhibitors in HIV patients on combination antiretroviral treatment. *AIDS* 20, 813-820 (2006)
101. Aukrust P., E. M. Aandahl, B. S. Skalhegg, I. Nordoy, V. Hansson, K. Tasken, S. S. Froland & F. Muller: Increased activation of protein kinase A type I contributes to the T cell deficiency in common variable immunodeficiency. *J Immunol* 162, 1178-1185 (1999)
102. Rahmouni S., E. M. Aandahl, M. Trebak, J. Boniver, K. Tasken & M. Moutschen: Increased cAMP levels and protein kinase (PKA) type I activation in CD4+ T cells and

B cells contribute to retrovirus-induced immunodeficiency of mice (MAIDS): a useful in vivo model for drug testing. *FASEB J* 15, 1466-1468 (2001)
103. Ledbetter J. A., M. Parsons, P. J. Martin, J. A. Hansen, P. S. Rabinovitch & C. H. June: Antibody binding to CD5 (Tp67) and Tp44 T cell surface molecules: effects on cyclic nucleotides, cytoplasmic free calcium, and cAMP-mediated suppression. *J Immunol* 137, 3299-3305 (1986)
104. Abrahamsen H., G. Baillie, J. Ngai, T. Vang, K. Nika, A. Ruppelt, T. Mustelin, M. Zaccolo, M. Houslay & K. Tasken: TCR- and CD28-mediated recruitment of phosphodiesterase 4 to lipid rafts potentiates TCR signaling. *J Immunol* 173, 4847-4858 (2004)
105. Houslay M. D. & D. R. Adams: PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochem J* 370, 1-18 (2003)
106. Manganiello V. C. & E. Degerman: Cyclic nucleotide phosphodiesterases (PDEs): diverse regulators of cyclic nucleotide signals and inviting molecular targets for novel therapeutic agents. *Thromb Haemost* 82, 407-411 (1999)
107. Conti M. & S. L. Jin: The molecular biology of cyclic nucleotide phosphodiesterases. *Prog Nucleic Acid Res Mol Biol* 63, 1-38 (1999)
108. Erdogan S. & M. D. Houslay: Challenge of human Jurkat T-cells with the adenylate cyclase activator forskolin elicits major changes in cAMP phosphodiesterase (PDE) expression by up-regulating PDE3 and inducing PDE4D1 and PDE4D2 splice variants as well as down-regulating a novel PDE4A splice variant. *Biochem J* 321 (Pt 1), 165-175 (1997)
109. Ekholm D., J. C. Mulloy, G. Gao, E. Degerman, G. Franchini & V. C. Manganiello: Cyclic nucleotide phosphodiesterases (PDE) 3 and 4 in normal, malignant, and HTLV-I transformed human lymphocytes. *Biochem Pharmacol* 58, 935-950 (1999)
110. Seybold J., R. Newton, L. Wright, P. A. Finney, N. Suttorp, P. J. Barnes, I. M. Adcock & M. A. Giembycz: Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T-cells and in human peripheral blood T-lymphocytes by 8-bromo-cAMP and Gs-coupled receptor agonists. Potential role in beta2-adrenoreceptor desensitization. *J Biol Chem* 273, 20575-20588 (1998)
111. Arp J., M. G. Kirchhof, M. L. Baroja, S. H. Nazarian, T. A. Chau, C. A. Strathdee, E. H. Ball & J. Madrenas: Regulation of T-cell activation by phosphodiesterase 4B2 requires its dynamic redistribution during immunological synapse formation. *Mol Cell Biol* 23, 8042-8057 (2003)
112. Soderling S. H. & J. A. Beavo: Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Op Cell Biol* 12, 174-179 (2000)
113. Spina D.: Theophylline and PDE4 inhibitors in asthma. *Curr Opin Pulm Med* 9, 57-64 (2003)
114. Giembycz M. A.: Cilomilast: a second generation phosphodiesterase 4 inhibitor for asthma and chronic obstructive pulmonary disease. *Expert Opin Investig Drugs* 10, 1361-1379 (2001)
115. Vang T., H. Abrahamsen, S. Myklebust, V. Horejsi & K. Tasken: Combined spatial and enzymatic regulation of Csk by cAMP and protein kinase a inhibits T cell receptor signaling. *J Biol Chem* 278, 17597-17600 (2003)
116. Luttrell L. M., S. S. Ferguson, Y. Daaka, W. E. Miller, S. Maudsley, G. J. Della Rocca, F. Lin, H. Kawakatsu, K. Owada, D. K. Luttrell, M. G. Caron & R. J. Lefkowitz: Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science* 283, 655-661 (1999)
117. Perry S. J., G. S. Baillie, T. A. Kohout, I. McPhee, M. M. Magiera, K. L. Ang, W. E. Miller, A. J. McLean, M. Conti, M. D. Houslay & R. J. Lefkowitz: Targeting of cyclic AMP degradation to beta 2-adrenergic receptors by beta-arrestins. *Science* 298, 834-836 (2002)
118. Baillie G. S., A. Sood, I. McPhee, I. Gall, S. J. Perry, R. J. Lefkowitz & M. D. Houslay: beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from Gs to Gi. *Proc Natl Acad Sci U S A* 100, 940-945 (2003)
119. MacKenzie S. J., G. S. Baillie, I. McPhee, C. MacKenzie, R. Seamons, T. McSorley, J. Millen, M. B. Beard, G. van Heeke & M. D. Houslay: Long PDE4 cAMP specific phosphodiesterases are activated by protein kinase A-mediated phosphorylation of a single serine residue in Upstream Conserved Region 1 (UCR1). *Br J Pharmacol* 136, 421-433 (2002)
120. Sette C. & M. Conti: Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. *J Biol Chem* 271, 16526-16534 (1996)
121. MacKenzie S. J., G. S. Baillie, I. McPhee, G. B. Bolger & M. D. Houslay: ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases - The involvement of COOH-terminal docking sites and NH2-terminal UCR regions. *J Biol Chem* 275, 16609-16617 (2000)
122. Whitehurst C. E., T. G. Boulton, M. H. Cobb & T. D. Geppert: Extracellular signal-regulated kinases in T cells. Anti-CD3 and 4 beta-phorbol 12-myristate 13-acetate-induced phosphorylation and activation. *J Immunol* 148, 3230-3237 (1992)
123. Li L., C. Yee & J. A. Beavo: CD3- and CD28-dependent induction of PDE7 required for T cell activation. *Science* 283, 848-851 (1999)
124. Yang G., K. W. McIntyre, R. M. Townsend, H. H. Shen, W. J. Pitts, J. H. Dodd, S. G. Nadler, M. McKinnon & A. J. Watson: Phosphodiesterase 7A-deficient mice have functional T cells. *J Immunol* 171, 6414-6420 (2003)
125. Acuto O., S. Mise-Omata, G. Mangino & F. Michel: Molecular modifiers of T cell antigen receptor triggering threshold: the mechanism of CD28 costimulatory receptor. *Immunol Rev* 192, 21-31 (2003)
126. Michel F., G. Attal-Bonnefoy, G. Mangino, S. Mise-Omata & O. Acuto: CD28 as a molecular amplifier extending TCR ligation and signaling capabilities. *Immunity* 15, 935-945 (2001)

Key Words: Immune system, cAMP, Csk, Lck, T cell, Lipid Raft, Review

Send correspondence to: Kjetil Taskén, M.D., Ph.D., Professor and Centre Director, The Biotechnology Centre of Oslo, University of Oslo, P.O. Box 1125, Blindern, N-0317 Oslo, Norway, Tel: 47-22840505, Fax: 47-22840506, E-mail: kjetil.tasken@biotek.uio.no