View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Allergology International (2002) **51**: 167–174

Review Article

T cell signaling: Protein kinase Cθ, the immunological synapse and characterization of SLAT, a novel T helper 2-specific adapter protein

Yoshihiko Tanaka and Amnon Altman

Division of Cell Biology, La Jolla Institute for Allergy and Immunology, San Diego, California, USA

Abstract

Triggering of the antigen-specific T cell receptor (TCR) can lead to various functional outcomes, such as activation and proliferation, anergy or cell death. This differential signaling is mainly determined by the quality and quantity of TCR signals, the nature of accessory signals and the differentiation/maturation status of the T cell. In this regard, T cell development and differentiation of the two major T helper (Th) subsets, namely Th1 and Th2 cells, can also be viewed as examples of differential signaling. In the present report, we review two T cell-selective signaling molecules (protein kinase C (PKC) θ and SLAT), which we have studied extensively and that appear to play important roles in the process of differential signaling. The novel PKC isoform PKCθ is selectively expressed in T lymphocytes and is essential for TCR-triggered activation of mature T cells via activation of the nuclear factor-κB and activator protein-1 pathways. Productive engagement of T cells by antigen-presenting cells (APC) results in recruitment of PKCθ to the T cell–APC contact area, the immunological synapse (IS), where it interacts with several signaling molecules to induce activation signals essential for productive T cell activation and interleukin-2 production. These events are associated with PKCθ translocation to membrane lipid rafts, which also localize to the IS. The Vav/Rac pathway promotes the recruitment of PKCθ to the IS or lipid rafts as well as its activation. SLAT is a novel adapter protein, which we isolated recently. It is selectively expressed in Th2 lineage cells, where it is found associated with the TCR-coupled protein tyrosine kinase ZAP-70. Our initial characterization of SLAT indicates that, by regulating the overall strength of TCR signaling, it may play an important role in differential signaling processes, which promote the differentiation and activation of allergy promoting and anti-inflammatory Th2 cells.

Key words: immune synapse, protein kinase C θ, SLAT, T cell receptor/ CD28, Th2.

Introduction

An effective immune response depends on the ability of specialized immunocytes to identify foreign molecules and respond by differentiation into mature effector cells. This tightly regulated process is mediated by a cell surface antigen recognition apparatus and a complex intracellular receptor-coupled signal-transducing machinery, which operate at high fidelity to discriminate self from non-self antigens.

The T cell receptor (TCR) is composed of two covalently bound polymorphic subunits, which provide antigen specificity, in association with at least four different types of invariant chains, which are essential for signal transduction. Activation of T lymphocytes requires sustained physical interaction of the TCR with a major histocompatibility complex (MHC)-presented peptide antigen. This interaction results in a temporal and spatial reorganization of multiple cellular elements at the T cell–antigen-presenting cell (APC) contact region, a specialized region referred to as the immunological synapse (IS)[1] or the supramolecular activation cluster (SMAC).[2]

T cell receptor engagement by a peptide/MHC complex is one essential signal ('signal 1') for T cell activation. However, productive T cell activation depends on an additional signal ('signal 2'), which can be provided by a number of costimulatory receptors. The major

Correspondence: Dr Amnon Altman, Division of Cell Biology, La Jolla Institute for Allergy and Immunology, San Diego, CA 92121, USA. Email: amnon@liai.org

Received 11 March 2002.

costimulatory signal for T cell activation is provided by interaction of the T cell surface molecule CD28 with its CD80/CD86 (B7-1/B7-2) ligands on APC.[3–6] This interaction plays an essential role in TCR-mediated interleukin (IL)-2 production. The nature of the CD28 costimulatory signal is relatively poorly defined. It may result from unique biochemical signals, such as activation of phosphatidylinositol 3-kinase (PI3K), and/or its ability to enhance, in a relatively non-specific manner, TCR signals, perhaps by facilitating formation of the IS and stabilizing it via its positive effect on lipid raft clustering.[7]

Studies during the past two decades revealed and characterized many types of effector molecules that play a role in the TCR-linked signal transduction machinery.[8] This work has led to the recognition of the critical role of multiple enzymes and adapter proteins in the early signaling events downstream of the engaged TCR. During the past 12 years, much work was centered on the role of reversible tyrosine phosphorylation and the related enzymes (i.e. protein tyrosine kinases and phosphatases) in regulating T cell activation. However, historically, the earliest studies on T cell activation have focused on the regulatory role of inositol phospholipid turnover and the resulting second messengers. These early studies[9] revealed that TCR engagement leads to phospholipase C (PLC)-γ1-mediated hydrolysis of membrane inositol phospholipids and subsequent production of inositol phosphates and diacylglycerol (DAG). These two second messengers stimulate, in turn, an elevation in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and activation of protein kinase C (PKC). Further support for the importance of these events in T cell activation was obtained using a combination of Ca^{2+} ionophore and phorbol ester tumor promoters (such as phorbol myristate acetate (PMA)), which mimicked TCR signals leading to full T cell activation resulting in IL-2 production and proliferation.[10–13]

The discovery of PKC as a lipid- and Ca^{2+}-dependent serine/threonine kinase, which serves as a cellular receptor for tumor-promoting phorbol ester,[14] implicated PKC in activation and mitogenesis of T cells. This was further substantiated by demonstrating that cellular depletion of PKC by prolonged treatment with phorbol esters, or PKC inhibitory drugs, significantly inhibited T cell activation.[15] During the same period, progress has been made in isolating the cDNA encoding different PKC enzymes.[16] This work revealed that PKC constitutes a family of multiple enzymes encoded by different genes, which have distinct biochemical properties, expression profiles and physiologic functions. However, the specific contribution of distinct PKC isoforms to TCR-coupled signaling pathways has not been resolved until very recently. This gap in our knowledge was largely due to the relatively slow progress in identifying specific physiologic substrates and functions of distinct PKC isoenzymes in T cells.

Recent studies have started to fill this gap by providing new information on PKCθ, which is now known to selectively mediate several essential functions in TCR-linked signaling leading to cell activation, differentiation and survival.

Properties and function of PKCθ

The discovery of PKC and its initial isolation and characterization revealed that this enzyme family is ubiquitously expressed and is also abundant in lymphoid tissues, peripheral blood mononuclear cells and leukemic cell lines.[17,18] Later studies demonstrated that PKC is the cellular receptor for phorbol ester tumor promoters,[19–21] providing a long sought after explanation for the observed effects of PMA on T cell mitogenesis.[22,23] In addition, both PKC-interacting phorbol esters and T cell proliferation-inducing agents (such as mitogenic lectins or antireceptor antibodies) induced a similar redistribution of PKC in T cells, characterized by its translocation from the cytosol to the particulate fraction.[24–30] Together with the observation that PMA binding upregulates PKC activity,[16] these results were consistent with the idea that PKC plays a key role in the activation response of T cells.

Cloning of several *PKC* genes led to the realization that these enzymes represent a large family encoded by distinct genes. Analysis of their tissue distribution demonstrated that different isoenzymes are expressed within individual cells and that many isoenzymes are expressed in a wide range of cell types and tissues. The finding that PKC activity is essential for TCR/CD3-induced T cell activation[15] led us to initiate a search for PKC isoforms that may play a specific role in T cell development and/or activation. These efforts led to the identification of a new member of the Ca^{2+}-independent novel PKC subfamily termed PKCθ.[31] Other investigators also cloned the corresponding mouse and human cDNA.[32,33] Chromosomal mapping located the human *PKCθ* gene to the short arm of chromosome 10 (10p15),[34] a region prone to mutations leading to T cell leukemia and lymphomas and T cell immunodeficiencies.[35,36]

Protein kinase C θ displays several unique properties that distinguish it from other T cell-expressed PKC enzymes and attest to its important role in T cell activation.[37,38]

First, it selectively activates the transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB).[39–42] These actions require integration of signals generated by the antigen-specific TCR and CD28, the major costimulatory receptor on T cells. These signals result in activation of the CD28 response element (RE) in the *IL-2* gene promoter.[40] Induction of CD28 RE is essential for productive T cell activation and secretion of IL-2, the major T cell growth factor. Indeed, in the absence of a CD28 costimulatory signal (and the resulting CD28 RE activation), T cells enter a stable state of unresponsiveness termed anergy.[43] Consistent with the important role of PKCθ in CD28 RE activation, the mature T cells of *PKCθ* gene-knockout mice display a severe defect in TCR/CD28-induced proliferation and IL-2 secretion, which can be traced to deficient NF-κB and AP-1 activation.[42] Importantly, PKCθ does not play any apparent role in thymic development.[42]

Second, PKCθ synergizes with calcineurin (Cn), a Ca^{2+}-activated phosphatase, to activate the transcription factor nuclear factor of activated T cells (NFAT) and the *IL-2* gene.[44,45] Third, PKCθ, but not other PKC isoforms, translocates to the site of cell contact between antigen-specific T cells and APC, the so-called IS,[35] where it colocalizes with the TCR in the central core of the T cell SMAC.[2,26] This event correlates with productive T cell activation leading to IL-2 production. Fourth, antigen stimulation induces translocation of PKCθ to specialized membrane microdomains or lipid rafts,[29] which are known to play an important role in T cell activation.[22,24] Furthermore, the lipid raft translocation of PKCθ is essential for its proper function during T cell activation.[29] Fifth, PKCθ also activates the c-Jun NH_2-terminal kinase (JNK) in T cells and synergizes with Cn to induce maximal activation.[44–47] However, the biologic significance of this pathway is unclear because JNK is not required for IL-2 production by naïve activated T cells[48] and JNK activation is intact in T cells from PKCθ-knockout mice.[42] These special properties of PKCθ are largely specified by a unique mechanism that regulates its cellular localization and activation (see below).

Protein kinase Cθ and the T cell synapse

T cell activation requires sustained TCR interaction with MHC-bound peptide antigen at the T cell–APC contact region. Productive interaction results in biochemical changes and reorganization of specific membrane domains, which lead to the formation of a highly ordered signaling complex at the contact site, the so called IS,[1] a term originally coined in reference to the polarized cytokine secretion by antigen-stimulated T cells.[17] Formation of a functional IS also involves the assembly of signaling complexes consisting of TCR, costimulatory accessory receptors (such as CD28, CD4/CD8 or lymphocyte function-associated antigen (LFA)-1) and intracellular signaling effector proteins,[1,2,18] reorganization of the actin cytoskeleton[18–20] and clustering of specialized membrane microdomains or lipid rafts.[21–24] A more detailed analysis of the T cell–APC contact region revealed compartmentalization of molecules in at least two distinct identifiable areas of the synapse, the so-called central SMAC (cSMAC) and peripheral SMAC (pSMAC).[2] While the cSMAC is characterized by clustering of TCR and MHC molecules on the T cell and APC surfaces, respectively, the pSMAC in these two cell types is enriched with LFA-1 integrins and their intercellular adhesion molecule-1 (ICAM-1) counterreceptors, respectively. The spatial organization and stability (or duration) of the IS determine the functional outcome of TCR engagement and underlie the fundamental phenomenon of differential T cell signaling.[25]

The initial findings linking PKCθ to the IS demonstrated that engagement of antigen-specific T cells by APC led to a rapid, stable and high-stoichiometry localization of PKCθ, but not other T cell-expressed PKC (βI, δ, ∈, η and ζ), to the T cell–APC contact site[26] and, more specifically, to the cSMAC.[2] This clustering correlated with the catalytic activation of PKCθ and it only occurred upon productive activation of T cells (i.e. upon exposure to APC that were fed with optimal antigen concentrations leading to efficient proliferation). In contrast, altered peptide ligands or low peptide concentrations that induced weak or no detectable proliferation did not promote PKCθ recruitment to the cSMAC.[26] Coclustering of talin and tubulin, and formation and reorientation of the microtubule-organizing center (MTOC), were also observed under these conditions. Subsequently, it became clear that signaling molecules on the inner side of the cell membrane also segregate into two non-overlapping regions characterized by PKCθ and Lck at the cSMAC, just below the TCR, and talin molecules in the peripheral zone, where they can directly interact with the LFA-1 cytoplasmic tail.[1,2]

The plasma membrane of many cell types, including T cells, contains glycosphingolipid-enriched membrane microdomains (GEM) or detergent-insoluble glycolipid (DIG) fractions, which are enriched in multiple

glycosyl-phosphatidylinositol (GPI)-anchored proteins. Because of their distinct biochemical and physical properties, these microdomains are relatively resistant to non-ionic detergents, characterized by low buoyant density, and, therefore, can be isolated by sucrose gradient centrifugation. They can also be identified *in vitro* or in intact cells by their selective ability to associate with the cholera toxin B (Ctx) subunit, which binds selectively to the lipid raft-enriched glycosphingolipid, GM1. These membrane microdomains are likely to correspond to the lipid rafts, which function as 'floating' platforms for the assembly of signaling complexes, following the engagement of specific cell surface receptors.[27,28] Distinct receptors or intracellular signaling molecules associate with lipid rafts in T cells either constitutively or following T cell activation and lipid raft integrity is important for productive T cell activation.[21–24]

A recent study addressed the relationship between lipid rafts and the IS with respect to the subcellular localization of PKCθ.[29] Anti-CD3 stimulation induced recruitment of a small amount of PKCθ to lipid rafts, an effect that was augmented by CD28 costimulation.[29] Moreover, TCR/CD28 costimulation of primary human T cells induced a simultaneous translocation of endogenous inhibitor of kappa B factor kinase (IKK) to the lipid rafts, where physical interaction with PKCθ was observed.[30] T cell receptor/CD28 crosslinking also increased the enzymatic activity of the lipid raft-residing PKCθ[29] and its ability to phosphorylate and activate IKKβ.[30] Stimulation of antigen-specific T cells by a peptide antigen presented by syngeneic APC induced simultaneous translocation and colocalization of PKCθ and Ctx-labeled membrane rafts at the T cell synapse.[29] Overexpression of truncated versions of PKCθ revealed that its translocation to rafts required the N-terminal regulatory domain. Nevertheless, the presence of functional catalytic domain of PKCθ in the lipid rafts was essential for the activation response. Thus, replacement of the PKCθ regulatory domain by a short sequence (seven amino acids) derived from the Lck amino terminus (which includes a membrane targeting sequence with myristoylation and acylation sites) allowed the localization of the chimeric PKCθ molecule to the rafts of non-stimulated T cells. However, lipid raft localization of this PKCθ form was insufficient for induction of T cell activation (as assessed by activation of NF-κB), which occurred only following cell stimulation with anti-CD28 antibodies plus PMA.[29]

Previous studies demonstrated that PKCθ is a target for phosphorylation by Lck in activated T cells.[31] Because both PKCθ and Lck localize to membrane rafts and are essential for the activation response, it was of interest to analyze whether Lck also affects PKCθ recruitment to membrane rafts. By comparing PKCθ cellular redistribution in activated wild-type versus Lck-deficient Jurkat T cells, Bi *et al*. found that Lck is essential for the inducible translocation of PKCθ to membrane rafts.[29] Clustering of PKCθ in the rafts was also dependent on the enzymatic activity of Lck and could be inhibited by the Lck-specific inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2). Furthermore, the lipid raft-residing PKCθ was associated with Lck, as observed by coimmunoprecipitation studies. In addition, the Lck-mediated tyrosine phosphorylation of PKCθ was dependent on the integrity of lipid rafts and was necessary for optimal induction of PKCθ-dependent biologic activities, such as NF-κB activation. However, it is possible that additional raft-residing effector molecules (rather than Lck only), which are involved in the T cell activation process, also regulate PKCθ activity and distribution. This notion is supported by recent findings demonstrating that intact ZAP-70 and SLP-76 are essential for activation of both PKCθ and its downstream effector molecule NF-κB[32] and, furthermore, that PKCθ cooperates with Akt/PKB to activate NF-κB in T cells.[33,34]

Regulation of PKCθ by the Vav/Rac pathway

Cytoskeletal components play critical roles in the regulation of T cell–APC contact and ensuing events leading to T cell activation. The actin cytoskeleton drives the assembly of large clusters of signaling molecules and formation of the IS.[18,19,35] Localization of PKCθ to the synapse and its selective involvement in early TCR/CD28 signaling events leading to T cell activation and proliferation raises questions regarding the mechanism that enables the selective recruitment of PKCθ to the T cell synapse. Unlike other T cell-expressed PKC enzymes, a fraction of cellular PKCθ associates with the cytoskeleton upon T cell activation.[35,36] In light of recent findings that the Vav/Rac pathway plays an important role in reorganization of the T cell actin cytoskeleton and in TCR capping,[49,50] Villalba *et al*. studied the potential role of the Vav/Rac pathway in regulating the recruitment of PKCθ to the membrane and its activation. It was found that Vav promoted the translocation of PKCθ from the cytosol to the membrane and cytoskeleton.[35] In addition, Vav induced PKCθ activation in a CD3/CD28 costimulation pathway that was dependent on Rac and actin

cytoskeleton reorganization. Furthermore, a TCR/CD28-coupled Vav signaling pathway that mediated the activation of JNK and the *IL-2* gene and upregulated CD69 expression was dependent on intact PKCθ function, because all these Vav-induced responses were inhibited by a dominant negative PKCθ mutant or by a selective PKCθ inhibitor.[35] These findings reveal that the Vav/Rac pathway promotes the recruitment of PKCθ to the T cell synapse and its activation. However, the identity of the protein (or lipid?) that actually carries PKCθ to the T cell synapse remains unknown. It is possible that PKCθ specifically binds to a cytoskeletal protein or to some scaffold protein that is associated with a component of the T cell cytoskeleton or synapse.

In this respect, it is important to note that PKCθ was identified as the major Ser/Thr kinase that phosphorylates moesin, a member of the ezrin-radixin-moesin (ERM) family of membrane-cytoskeleton linking proteins.[51] Phosphorylation occurred on Thr^{558} the *in vivo* phosphorylation site of moesin within its conserved actin-binding domain, suggesting that PKCθ may also regulate actin-based organization of membrane components via its effect on moesin. This effect may be relevant to synapse formation in APC-engaged T cells. However, overexpression of active PKCθ alone did not induce significant actin polymerization in T cells.[35] Although chemokine stimulation, which induces T cell polarization, causes moesin to translocate to the uropod (i.e. the T cell pole opposite the IS[52]), the localization of moesin in antigen-stimulated T cells has not been examined in detail.

SWAP-70-LIKE ADAPTER OF T CELLS (SLAT)

Introduction: The Th1/Th2 paradigm

T helper cells play a central role in the immune response via direct cell–cell contact or secretion of multiple immunoregulatory cytokines. The division of Th cells into two subsets based on their pattern of cytokine production was first recognized among mouse $CD4^+$ T cell clones[53] and later among human T cells.[54] Mouse Th1 cells secrete IL-2, interferon (IFN)-γ and lymphotoxin (LT), whereas Th2 cells produce IL-4, -5, -6, -9, -10 and -13. Human Th subsets produce similar cytokine profiles, usually in a less tightly restricted manner.[55,56] The Th1 and Th2 subsets are thought to derive from a single precursor naïve T cell (Th0 subset). Cross-regulation among Th1 and Th2 cells is mediated by cytokines, which inhibit the differentiation and function of the reciprocal subset. Although this simple division of Th cells into two defined subsets underestimates the complexity of the system, it is clear that many antigen-specific T cell clones and *in vivo* immune responses display a clear dichotomy between Th1- and Th2-selective cytokine responses. This dichotomy underlies an important functional division. Thus, activated Th1 cells mediate primarily cell-mediated inflammatory responses (e.g. delayed-type hypersensitivity). The Th2 cells function mainly as helper cells for antibody responses (particularly IgE) and enhance eosinophil proliferation and function.[55,56]

The importance of Th1 and Th2 cells in the normal physiology and pathology of the immune system is underscored by many studies documenting the predominance of a given subset in infection, autoimmune diseases, transplant rejection and allergic diseases.[56] Interleukin-4 and -5 (but not IFN-γ) are detected in late-phase cutaneous allergic reactions, allergen-specific T cells display a strong Th2 bias and Th2 cytokines in bronchial lavage fluid and eosinophilia are found in atopic asthma. These and other findings have led to the notion that Th2-derived cytokines play a central role in the pathophysiology of allergy and asthma.[57] Thus, Th2 cells and their characteristic cytokines represent an important drug development target for the treatment of these diseases. In this regard, effective drug design and development require a detailed understanding of the factors that determine both the commitment and differentiation of Th0 cells into the Th1/Th2 subset and the regulation/activation of the two mature subsets.

Signaling in Th1 compared with Th2 subsets

The well-documented production of distinct cytokines by committed Th1 and Th2 cells, which respond to the same antigen, suggests that qualitative and/or quantitative differences in signals delivered by the TCR and/or costimulatory receptors upon engagement by peptide/APC account for this differential cytokine production. Thus, activation of Th1/Th2 cells, as well as their differentiation from naïve cells, can be viewed as cases of differential TCR signaling. The TCR affinity for its ligand, the type of APC or the nature of the costimulatory signals have all been shown to influence the pattern of Th differentiation and cytokine production. Most of the progress in this regard was made in the area of transcriptional regulation of cytokine production.[58,59] Thus, the transcription factors GATA-3 and c-Maf are highly expressed in Th2 cells, but not Th1 cells, and

induce high levels of IL-4 and IL-5. Conversely, T-bet is expressed in differentiated Th1 cells but not Th2 cells and can transactivate the IFN-γ promoter.

The majority of studies on these factors to date has focused on their induction in response to cytokine receptor signals. However, these events occur downstream of the initial regulation of Th1 and Th2 cells, which must rely on production of IL-4, IFN-γ and IL-12 in response to TCR and costimulatory signals in the first place. As an example, Th2 cells can be generated in the complete absence of the IL-4R or its signaling intermediate signal transducers and activators of transcription (STAT)-6[60–62] and Th1 cells can be generated in the absence of the IL-12R signaling intermediate STAT-4.[63] This suggests that commitment to the Th1 or Th2 differentiation pathways is decided before cytokine receptor signaling occurs and that the latter may serve primarily to amplify the number of Th1 and Th2 cells that are generated and/or survive. Consistent with this notion, recent studies demonstrated that TCR signaling intensities regulate the differentiation of naïve Th cells into Th1 or Th2 phenotypes.[64,65] Other studies implicated a differential role for TCR proximal signaling pathways, such as non-receptor protein tyrosine kinase (PTK), PKC, Cn and mitogen-activated protein kinase in Th subset differentiation and activation.[58,59] However, much remains to be learned about differences in early membrane proximal TCR signals associated with Th differentiation and/or differential cytokine production by committed Th subsets.

SLAT

In an attempt to identify novel Th2-specific signaling proteins, we performed a differential display analysis[66] of cDNA obtained from pigeon cytochrome C (PCC)-specific AD10 TCR-transgenic T cells, which were cultured for 4 days under defined conditions leading to polarized Th1 or Th2 cells. This polarization was confirmed by intracellular cytokine staining of IFN-γ and IL-4, respectively. We focused our attention on one cDNA, which hybridized to an approximate 1.8 kb transcript expressed in Th2, but not Th1, cells. The rapid amplification of cDNA ends (RACE) method was used to extend this partial cDNA and obtain a complete reading frame encoding a putative protein of 630 amino acids. Sequence analysis revealed that the cDNA corresponds to a novel gene. Sequence comparison of the putative protein with the GenBank database revealed identities of 45% with murine SWAP-70.[67] The domain structure of this novel protein is very similar to that of SWAP-70. SWAP-70 is a novel protein of 70 kDa, which is expressed only in B lymphocytes that have been induced to switch to various Ig isotypes and in switching B cell lines.[67] B cells of SWAP-70-deficient mice are more sensitive to γ-irradiation than B cells of wild-type mice and they display defects in CD40- and lipopolysaccharide-dependent switching to the IgE isotype, but not to other isotypes.[68] These results suggest that SWAP-70 serves a specific role in the CD40-dependent signaling pathway leading to IgE production and, by inference, in regulating the allergic response. SLAT mRNA expression correlated with Th2 differentiation and the corresponding protein was preferentially expressed in Th2 membrane, in T cell lines and in the thymus. SLAT inducibly associated with ZAP-70 in antigen-activated Th2 cells and agonist stimulation of antigen-specific Th2 cells induced its translocation to the central region (cSMAC?) of the T cell synapse. Finally, transient overexpression of SLAT in Jurkat T cells stimulated the anti-CD3-induced activation of an IL-4 gene and, conversely, reduced the induction of an IFN-γ reporter reporter gene. Consistent with this result, retrovirus-mediated SLAT expression reduced the fraction of IFN-γ-producing (Th1) primary T cells, but increased the number of IL-4-producing differentiated Th2 cells. Based on these homologies and the respective expression profiles, we named the novel cDNA and its putative protein product SWAP-70-like adapter of T cells (SLAT). We propose that SLAT is the T cell homolog of B cell-specific SWAP-70. Thus, SLAT is a novel candidate for mediating early TCR signaling events in Th2 lineage cells.

Acknowledgments

We thank our many colleagues who contributed to the work on PKCθ and SLAT in our laboratories and provided advice. The work in our laboratories was supported, in part, by grants from the National Institutes of Health (CA35299 and GM50819 to AA) and the Uehara Memorial Foundation grant (to YT). This is publication number 499 from the La Jolla Institute for Allergy and Immunology.

References

1 Grakoui A, Bromley SK, Sumen C *et al*. The immunological synapse: A molecular machine controlling T cell activation. *Science* 1999; **285**: 221–7.

2 Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 1998; **395**: 82–6.

3 Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu. Rev. Immunol.* 1996; **14**: 233–58.

4 Greenfield EA, Nguyen KA, Kuchroo VK. CD28/B7 costimulation: A review. *Crit. Rev. Immunol.* 1998; **18**: 389–418.

5 Powell JD, Ragheb JA, Kitagawa-Sakakida S, Schwartz RH. Molecular regulation of interleukin-2 expression by CD28 co-stimulation and anergy. *Immunol. Rev.* 1998; **165**: 287–300.

6 Chambers CA, Allison JP. Costimulatory regulation of T cell function. *Curr. Opin. Cell. Biol.* 1999; **11**: 203–10.

7 Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A. T lymphocyte costimulation mediated by reorganization of membrane microdomains. *Science* 1999; **283**: 680–2.

8 Weiss A, Littman D. Signal transduction by lymphocyte antigen receptors. *Cell* 1994; **76**: 263–74.

9 Altman A, Coggeshall KM, Mustelin T. Molecular events mediating T cell activation. *Adv. Immunol.* 1990; **48**: 227–360.

10 Isakov N, Altman A. Tumor promoters in conjunction with calcium ionophores mimic antigenic stimulation by reactivation of alloantigen-primed murine T lymphocytes. *J. Immunol.* 1985; **135**: 3674–80.

11 Kaibuchi K, Takai Y, Nishizuka Y. Protein kinase C and calcium ion in mitogenic response of macrophage-depleted human peripheral lymphocytes. *J. Biol. Chem.* 1985; **260**: 1366–9.

12 Truneh A, Albert F, Golstein P, Schmitt-Verhulst AM. Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. *Nature* 1985; **313**: 318–20.

13 Truneh A, Albert F, Golstein P, Schmitt-Verhulst AM. Calcium ionophore plus phorbol ester can substitute for antigen in the induction of cytolytic T lymphocytes from specifically primed precursors. *J. Immunol.* 1985; **135**: 2262–7.

14 Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* 1995; **9**: 484–96.

15 Altman A, Mustelin T, Coggeshall KM. T lymphocyte activation: A biological model of signal transduction. *Crit. Rev. Immunol.* 1990; **10**: 347–91.

16 Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* 1995; **9**: 484–96.

17 Paul WE, Seder RA. Lymphocyte responses and cytokines. *Cell* 1994; **76**: 241–51.

18 Wulfing C, Davis MM. A receptor/cytoskeletal movement triggered by costimulation during T cell activation. *Science* 1998; **282**: 2266–9.

19 Penninger JM, Crabtree GR. The actin cytoskeleton and lymphocyte activation. *Cell* 1999; **96**: 9–12.

20 Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: Molecular hardware for T cell signaling. *Nat. Immunol.* 2000; **1**: 23–9.

21 Horejsi V, Drbal K, Cebecauer M *et al.* GPI-microdomains: A role in signalling via immunoreceptors. *Immunol. Today* 1999; **20**: 356–61.

22 Xavier R, Seed B. Membrane compartmentation and the response to antigen. *Curr. Opin. Immunol.* 1999; **11**: 265–9.

23 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.

24 Bi K, Altman A. Membrane lipid microdomains and the role of PKCtheta in T cell activation. *Semin. Immunol.* 2001; **13**: 139–46.

25 Dustin ML, Chan AC. Signaling takes shape in the immune system. *Cell* 2000; **103**: 283–94.

26 Monks CR, Kupfer H, Tamir I, Barlow A, Kupfer A. Selective modulation of protein kinase C-theta during T-cell activation. *Nature* 1997; **385**: 83–6.

27 Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* 1997; **387**: 569–72.

28 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.

29 Bi K, Tanaka Y, Coudronniere N *et al.* Antigen-induced translocation of PKC-theta to membrane rafts is required for T cell activation. *Nat. Immunol.* 2001; **2**: 556–63.

30 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.

31 Liu Y, Witte S, Liu YC, Doyle M, Elly C, Altman A. Regulation of protein kinase Ctheta function during T cell activation by Lck-mediated tyrosine phosphorylation. *J. Biol. Chem.* 2000; **275**: 3603–9.

32 Herndon TM, Shan XC, Tsokos GC, Wange RL. ZAP-70 and SLP-76 regulate protein kinase C-theta and NF-kappa B activation in response to engagement of CD3 and CD28. *J. Immunol.* 2001; **166**: 5654–64.

33 Kane LP, Andres PG, Howland KC, Abbas AK, Weiss A. Akt provides the CD28 costimulatory signal for up-regulation of IL-2 and IFN-gamma but not Th2 cytokines. *Nat. Immunol.* 2001; **2**: 37–44.

34 Bauer B, Krumbock N, Fresser F *et al.* Complex formation and cooperation of protein kinase C theta and Akt1/protein kinase B alpha in the NF-kappa B transactivation cascade in Jurkat T cells. *J. Biol. Chem.* 2001; **276**: 31 627–34.

35 Dustin ML, Shaw AS. Costimulation: Building an immunological synapse. *Science* 1999; **283**: 649–50.

36 Meller N, Liu YC, Collins TL *et al.* Direct interaction between protein kinase C theta (PKC theta) and 14–3–3 tau in T cells: 14–3–3 overexpression results in inhibition of PKC theta translocation and function. *Mol. Cell. Biol.* 1996; **16**: 5782–91.

37 Altman A, Isakov N, Baier G. Protein kinase Ctheta. A new essential superstar on the T-cell stage. *Immunol. Today* 2000; **21**: 567–73.

38 Isakov N, Altman A. Protein kinase C-theta in T cell activation. *Annu. Rev. Immunol.* 2002; **20**: 761–94.
39 Baier-Bitterlich G, Uberall F, Bauer B *et al.* Protein kinase C-theta isoenzyme selective stimulation of the transcription factor complex AP-1 in T lymphocytes. *Mol. Cell. Biol.* 1996; **16**: 1842–50.
40 Coudronniere N, Villalba M, Englund N, Altman A. NF-kappa B activation induced by T cell receptor/CD28 costimulation is mediated by protein kinase C-theta. *Proc. Natl Acad. Sci. USA* 2000; **97**: 3394–9.
41 Lin X, O'Mahony A, Mu Y, Geleziunas R, Greene WC. Protein kinase C-theta participates in NF-kappaB activation induced by CD3-CD28 costimulation through selective activation of IkappaB kinase beta. *Mol. Cell. Biol.* 2000; **20**: 2933–40.
42 Sun Z, Arendt CW, Ellmeier W *et al.* PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. *Nature* 2000; **404**: 402–7.
43 Rudd CE. Upstream–downstream. CD28 cosignaling pathways and T cell function. *Immunity* 1996; **4**: 527–34.
44 Werlen G, Jacinto E, Xia Y, Karin M. Calcineurin preferentially synergizes with PKC-theta to activate JNK and IL-2 promoter in T lymphocytes. *EMBO J.* 1998; **17**: 3101–11.
45 Ghaffari-Tabrizi N, Bauer B, Villunger A *et al.* Protein kinase Ctheta, a selective upstream regulator of JNK/SAPK and IL-2 promoter activation in Jurkat T cells. *Eur. J. Immunol.* 1999; **29**: 132–42.
46 Avraham A, Jung S, Samuels Y, Seger R, Ben-Neriah Y. Co-stimulation-dependent activation of a JNK-kinase in T lymphocytes. *Eur. J. Immunol.* 1998; **28**: 2320–30.
47 Villalba M, Coudronniere N, Deckert M, Teixeiro E, Mas P, Altman A. A novel functional interaction between Vav and PKCtheta is required for TCR-induced T cell activation. *Immunity* 2000; **12**: 151–60.
48 Dong C, Yang DD, Tournier C *et al.* JNK is required for effector T-cell function but not for T-cell activation. *Nature* 2000; **405**: 91–4.
49 Fischer KD, Kong YY, Nishina H *et al.* Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. *Curr. Biol.* 1998; **8**: 554–62.
50 Holsinger LJ, Graef IA, Swat W *et al.* Defects in actin-cap formation in Vav-deficient mice implicate an actin requirement for lymphocyte signal transduction. *Curr. Biol.* 1998; **8**: 563–72.
51 Pietromonaco SF, Simons PC, Altman A, Elias L. Protein kinase C-theta phosphorylation of moesin in the actin-binding sequence. *J. Biol. Chem.* 1998; **273**: 7594–603.
52 Serrador JM, Alonso-Lebrero JL, del Pozo MA *et al.* Moesin interacts with the cytoplasmic region of intercellular adhesion molecule-3 and is redistributed to the uropod of T lymphocytes during cell polarization. *J. Cell. Biol.* 1997; **138**: 1409–23.
53 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986; **136**: 2348–57.
54 Del Prete GF, De Carli M, Mastromauro C *et al.* Purified protein derivative of *Mycobacterium tuberculosis* and excretory–secretory antigen(s) of *Toxocara canis* expand *in vitro* human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.* 1991; **88**: 346–50.
55 Fitch FW, McKisic MD, Lancki DW, Gajewski TF. Differential regulation of murine T lymphocyte subsets. *Annu. Rev. Immunol.* 1993; **11**: 29–48.
56 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* 1996; **17**: 138–46.
57 Anderson GP, Coyle AJ. TH2 and 'TH2-like' cells in allergy and asthma: Pharmacological perspectives. *Trends Pharmacol. Sci.* 1994; **15**: 324–32.
58 Glimcher LH, Singh H. Transcription factors in lymphocyte development: T and B cells get together. *Cell* 1999; **96**: 13–23.
59 Murphy KM, Ouyang W, Farrar JD *et al.* Signaling and transcription in T helper development. *Annu. Rev. Immunol.* 2000; **18**: 451–94.
60 Dent AL, Hu-Li J, Paul WE, Staudt LM. T helper type 2 inflammatory disease in the absence of interleukin 4 and transcription factor STAT6. *Proc. Natl Acad. Sci. USA* 1998; **95**: 13 823–8.
61 Finkelman FD, Morris SC, Orekhova T *et al.* Stat6 regulation of *in vivo* IL-4 responses. *J. Immunol.* 2000; **164**: 2303–10.
62 Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the *in vivo* or *in vitro* development of CD4+ lymphocytes with a Th2 cytokine profile. *J. Immunol.* 2000; **164**: 3047–55.
63 Kaplan MH, Wurster AL, Grusby MJ. A signal transducer and activator of transcription (Stat) 4-independent pathway for the development of T helper type 1 cells. *J. Exp. Med.* 1998; **188**: 1191–6.
64 Constant S, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J. Exp. Med.* 1995; **182**: 1591–6.
65 Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A. The effect of antigen dose on CD4+ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model. *J. Exp. Med.* 1995; **182**: 1579–84.
66 Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 1992; **257**: 967–71.
67 Borggrefe T, Wabl M, Akhmedov AT, Jessberger R. A B-cell-specific DNA recombination complex. *J. Biol. Chem.* 1998; **273**: 17 025–35.
68 Borggrefe T, Keshavarzi S, Gross B, Wabl M, Jessberger R. Impaired IgE response in SWAP-70-deficient mice. *Eur. J. Immunol.* 2001; **31**: 2467–75.