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The T-cell-specific adapter protein family: TSAd, ALX, and SH2D4A/SH2D4B

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Summary: Adapter proteins play key roles in intracellular signal transduction through complex formation with catalytically active signaling molecules. In T lymphocytes, the role of several different types of adapter proteins in T-cell antigen receptor signal transduction is well established. An exception to this is the family of T-cell-specific adapter (TSAd) proteins comprising of TSAd, adapter protein of unknown function (ALX), SH2D4A, and SH2D4B. Only recently has the function of these adapters in T-cell signal transduction been explored. Here, we discuss advances in our understanding of the role of this family of adapter proteins in T cells. Their function as regulators of signal transduction in other cell types is also discussed.

Keywords: *adapter proteins, protein tyrosine kinases, signal transduction, T lymphocytes, autoimmunity, knockout mice*

Introduction

Adapter proteins play central roles in intracellular signal transduction pathways initiated by different cell surface receptors in response to ligand recognition (1–3). Adapter proteins lack catalytic activity but instead usually contain one or more modular-binding domains or recognition sequences for modular-binding domains of other signaling proteins. As such, adapter proteins participate in the formation of signaling complexes with catalytically active molecules, resulting in direct or indirect modulation of catalytic activity and/or localization of catalytically active molecules to substrates. Testimony to the importance of adapter proteins in receptor signal transduction is the finding that gene-targeted mice that lack expression of specific adapter proteins frequently show signs of disease and disturbances of tissue homeostasis often associated with early mortality.

In T lymphocytes, the function of several different adapter proteins such as growth receptor-bound-2 (Grb-2), Grb-2-related adapter protein-2 (GRAP-2), Src homology-2 (SH2)-domain-containing leukocyte protein of 76 kDa (SLP-76), Fyn-binding protein (FYB), and linker of activated T cells (LAT) has been extensively characterized (4–8). In contrast,

adapter proteins belonging to the T-cell-specific adapter (TSAd) protein family have been less explored. This family comprises of TSAd itself (also known as SH2D2A), adapter protein of unknown function (ALX or HSH2D), SH2D4A, and SH2D4B (9). In this article, we review current knowledge relating to the function of these adapters in T cells. In addition, a role for these adapters in other cell types is discussed.

Identification and expression of TSAd family adapter proteins

TSAd

TSAd was first identified in humans as part of a subtractive cDNA library screen in which cDNA from peripheral blood $CD8^+$ T cells activated with CD3 plus CD28 monoclonal antibodies (mAbs) was subtracted using cDNA prepared from the Jurkat human T-cell leukemia cell line (10). From this screen, an open reading frame (ORF) was identified that encoded a 389-amino acid protein that resembled a typical intracellular adapter. Sequence inspection revealed a protein without any recognizable catalytic activity but instead an SH2 domain located in the center of the linear sequence with predicted affinity for protein phosphotyrosine (*Fig.* 1). In addition, in the region of the protein carboxyl to the SH2 domain, there are five tyrosine residues, four of which are contained within consensus motifs for phosphorylation by protein tyrosine kinases (PTKs), plus several proline-rich regions. Potentially, therefore, these sequences could serve as docking sites for SH2 or protein tyrosine-binding domain (PTB)-containing or SH3 domain-containing proteins, respectively (11).

Initial Northern blot analysis of different human tissues revealed that TSAd expression was restricted to thymus, spleen, and peripheral blood T lymphocytes (10). Furthermore, TSAd was not expressed in resting T cells but rather was induced in both $CD4^+$ and $CD8^+$ T cells following their activation with phorbol esters, phytohemagglutinin, or CD3 plus CD28 mAbs, consistent with the nature of the original subtractive cDNA library screen (10). The kinetics of induced expression were fairly rapid in that transcripts were detected within hours and reached a plateau by 1 day after stimulation. Western blot studies confirmed that the 52-kDa TSAd protein was not expressed in resting T cells but was induced upon T-cell activation with kinetics consistent with the increased RNA expression (12). Such studies also revealed synergism between CD3 and CD28 with regards to the induction of TSAd protein. Expression of TSAd in human T cells appears to be regulated in part by a cyclic adenosine monophosphate (AMP) response element contained within the promoter of the TSAd gene (13). However, activation of the cyclic AMP signal transduction pathway alone is not sufficient for induction of TSAd protein expression. Thus, a cyclic AMP analog, although able to induce TSAd mRNA, is unable to induce TSAd protein expression (14). Presumably, therefore, CD3/CD28 engagement additionally results in the activation of pathways that allow efficient translation of TSAd mRNA and/or promote stability of TSAd protein.

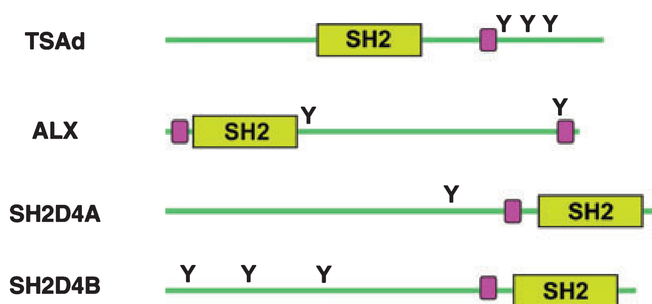

***Fig. 1.* TSAd (T-cell-specific adapter) family adapter proteins.** Positions of SH2 domains within linear sequences of each adapter are indicated. Proline-rich stretches that are conserved between mouse and human are shown in purple. Conserved tyrosine residues in consensus phosphorylation motifs (NetPhos program) are also shown.

Subsequent to the identification of TSAd in humans, TSAd was identified in mice by two independent groups, both using yeast-hybrid screening technology. Choi *et al.* (15) used a bait protein comprising of the SH2 domain plus kinase domain of the Src family PTK resting lymphocyte kinase (RLK). Rajagopal *et al.* (16) used the Tec-family PTK RLK as a bait. TSAd was found to interact with both types of bait protein and also another Tec family PTK, interleukin-2-inducible T cell kinase (ITK), hence the synonyms for TSAd, LCK-associated adapter (LAD) and RLK/ITK-binding protein (RIBP), respectively. Murine TSAd is a 366-amino acid protein that like human TSAd contains a single SH2 domain (*Fig.* 1). Four out of the five tyrosine residues found in the carboxyl region of human TSAd are conserved in the carboxyl region of mouse TSAd as is one of the proline-rich stretches. Interaction of TSAd with LCK and RLK in yeast was shown to require a catalytically active kinase domain in both cases. In addition, for the interaction with LCK, binding was shown to be abolished by a point mutation of the LCK SH2 domain that prevented protein phosphotyrosine recognition by this domain. These findings suggested that the mechanism of interaction of TSAd with PTKs involved PTK-mediated phosphorylation of TSAd carboxyl region tyrosine residues followed by PTK SH2 domain recognition of the same tyrosine residues.

Similar to human TSAd, murine TSAd was initially reported to be relatively restricted in expression to T cells and natural killer (NK) cells. Northern blot analysis revealed strong

expression in thymus, spleen, and peripheral blood mononuclear cells (15, 16). Transcripts were detected in T-helper 2 (Th2) and NK cell lines and in CD3 mAb-stimulated Th1 cells. In the latter case, transcripts were induced as soon as 2 h after stimulation (16). Induced expression of TSAd in murine T cells is consistent, therefore, with findings in human T cells. However, particularly in mouse T cells, TSAd protein does appear to be expressed at functionally significant levels prior to activation (15, 17). Furthermore, TSAd also appears to be expressed at functionally significant levels in some other primary cell types in mice including lung epithelial cells, blood vessel endothelial cells, and mature B cells (15, 18–20).

ALX

ALX was first identified as part of a search of databases for proteins with SH2 domains with significant sequence similarity to the SH2 domain of the c-ABL PTK (21). In humans, one such novel 353-amino acid residue protein was identified that contained a single SH2 domain at its amino-terminus (*Fig.* 1). A homologous 364-amino acid residue protein was also identified in mice. The SH2 domains of human and murine ALX show some similarity to the SH2 domain of c-Abl but in fact are more homologous to the SH2 domains of TSAd, SH2D4A, and SH2D4B (9). Aside from the SH2 domain, ALX shows little sequence similarity with TSAd, SH2D4A, or SH2D4B. However, like TSAd, outside of the SH2 domain, ALX contains proline-rich stretches and tyrosine residues in consensus phosphorylation motifs. There are two proline-rich stretches and two such tyrosine residues that are conserved between mouse and human ALX.

ALX was initially named HSH2 for hematopoietic SH2 protein owing to its restricted expression in this lineage. Analyses of mRNA expression revealed transcripts in peripheral blood leukocytes, thymus, and spleen, but not a number of examined non-hematopoietic tissues (21). More detailed analysis at both the mRNA and protein levels has shown that ALX is expressed in both T and B cells (20, 22–24). However, in contrast to TSAd, ALX expression is not increased in T cells (or B cells) in response to activating stimuli.

SH2D4A/SH2D4B

Further searches of protein databanks reveal two other proteins, SH2D4A and SH2D4B, which contain SH2 domains with significant homology to the SH2 domains of TSAd and ALX (*Fig.* 1). The SH2 domains of SH2D4A and SH2D4B are located at the carboxyl ends of the proteins and show the highest degree of homology to one another. In addition, there is significant homology between SH2D4A and SH2D4B in parts of the amino-terminal region (although there is no homology of this region to regions outside of the SH2 domains of TSAd and ALX). The amino-terminal region of SH2D4A contains a conserved tyrosine residue in a phosphorylation consensus motif and a proline-rich stretch near the SH2 domain. The amino-terminal region of SH2D4B contains three conserved tyrosine residues in consensus phosphorylation motifs and a proline-rich stretch located in the same position as that found in SH2D4A.

Analysis of expressed sequence tag and public microarray databases indicate that SH2D4A is expressed at low levels in a variety of human and murine tissues. With the use of a specific polyclonal antiserum, Lapinski *et al.* (25) demonstrated that in quiescent human T cells, the 52-kDa SH2D4A protein is expressed poorly but, like TSAd, is induced in response to CD3 plus CD28 mAb activation. However, this inducibility is not observed in murine T cells where the 48-kDa SH2D4A protein is found prior to activation. Other hematopoietic cell types in which SH2D4A has been found to be expressed include B cells, macrophages, and dendritic cells. In these cell types, SH2D4A is expressed constitutively and is not increased in response to activating stimuli (25). One report has documented expression of SH2D4A in podocytes of kidney glomeruli (26).

Less is known about the expression of SH2D4B. Database analyses indicate a rare transcript in hematopoietic and non-hematopoietic tissues. However, no information on protein expression is yet available.

TSAd family adapter proteins and T-cell signal transduction

TSAd

As a first approach to examine the function of TSAd in T cells, several groups examined the influence of overexpression of TSAd upon T-cell receptor (TCR)-induced activation of the promoter for the intereukin-2 (IL-2) cytokine in T-cell lines *in vitro*. However, conflicting results were obtained. Sunvold *et al.* (12) reported that overexpression of TSAd in Jurkat cells inhibited IL-2 promoter activity induced by the combination of CD3 mAb and phorbol ester. In contrast, Marti *et al.* (27) found that TSAd overexpression in Jurkat enhanced IL-2 promoter activity in response to the same stimulus as well as in response to CD3 plus CD28 mAb stimulation. Similarly, in murine EL-4 T-lymphoma cells, Choi *et al.* (15) found that overexpression of murine TSAd enhanced IL-2 promoter activity. It is possible that these discrepancies relate to the level of

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expression of TSAd in each instance, with a high level of expression causing inhibition and more modest expression resulting in augmentation of promoter activity. Whether such differences are of physiological relevance is unclear. On the one hand, augmented IL-2 promoter activity suggests a positive regulatory role for TSAd in T cells, and this role is consistent with the finding that T cells from TSAd-deficient mice synthesize reduced quantities of cytokines in response to TCR stimulation (see the following paragraphs). On the other hand, it is possible that TSAd may additionally perform a negative regulatory role during TCR signal transduction, perhaps when expressed at higher levels at later time points after the initiation of T-cell activation.

Following up on the identification of TSAd as an interaction partner of LCK in yeast-hybrid systems, different groups have examined physical and functional interactions between TSAd and LCK in T cells. LCK is one of the first PTKs to become activated during the course of TCR signal transduction (1, 28). Coengagement of the TCR and CD4 or CD8 coreceptors is thought to result in local aggregation of LCK, leading to phosphorylation of a positive regulatory tyrosine residue (Y394) contained in the LCK kinase domain with a resultant increase in LCK kinase activity. This activated LCK then phosphorylates tyrosine residues contained within immunoreceptor tyrosine-based activation motifs (ITAMs) present in the cytoplasmic tails of the TCR signaling chains, CD3 and TCR-ζ. The phosphorylated ITAMs are recognized by the SH2 domains of the SYK family PTKζ-associated protein of 70 kDa (ZAP70), which is thus recruited to the TCR signaling complex where it is juxtaposed to LCK and activated as a result of LCK-mediated phosphorylation. Subsequently, ZAP70 phosphorylates tyrosine residues on the LAT transmembrane adapter protein that then triggers signaling pathways that culminate in the activation of nuclear factor of activated T cells (NFAT), NFκB, and activator protein-1 (AP-1) transcription factors. Together, these transcription factors drive expression of numerous genes involved in T-cell activation including cytokines such as IL-2.

With this backdrop, an ability of TSAd to engage and modulate LCK kinase activity directly or indirectly could provide a basis for its ability to regulate TCR-induced cytokine synthesis. Physical interaction between TSAd and LCK has been confirmed in T-cell lines and primary T cells both between transfected and cell endogenous proteins (15, 17, 29–31). In general, physical association is not apparent in resting T cells but is induced rapidly in response to TCR engagement. Extensive mutational analyses performed *in vitro*, in yeast, and in T-cell lines have revealed at least two different mechanisms by which TSAd interacts with LCK (15, 17, 29–31). First, as discussed previously, the LCK SH2 domain binds to phosphorylated tyrosine residues in the TSAd carboxyl region. In transfected T-cell lines, as in yeast, LCK itself is able to phosphorylate these tyrosine residues and it is likely that cell endogenous LCK performs this role during the course of T-cell activation. Thus, the kinase activity of LCK promotes this form of interaction. The second type of interaction involves LCK SH3 domain recognition of the conserved proline-rich stretch present in the TSAd carboxyl tail. As LCK does not interact with TSAd in quiescent T cells, this form of interaction must also be induced. Potentially, this could involve tyrosine phosphorylation-mediated exposure of the proline-rich stretch in the TSAd carboxyl region and/or induced accessibility of the LCK SH3 domain during the course of T-cell activation (see subsequent paragraphs). Finally, the TSAd SH2 domain has been shown to mediate a weak form of interaction with the LCK kinase domain. In this case, the target tyrosine residue in LCK appears to be the Y394-positive regulatory tyrosine residue of the LCK kinase domain.

Some studies have reported an inhibitory effect of overexpressed TSAd upon TCR-mediated activation of LCK in Jurkat (12, 29–31). This has been inferred from the ability of overexpressed TSAd to: (i) block downstream signaling events that are known to be dependent upon LCK activation such as ZAP70 and LAT phosphorylation and activation of phospholipase C-γ (PLC-γ); (ii) increase the phosphorylation of LCK upon a carboxy-terminal negative regulatory tyrosine residue (Y505) to a greater degree than it is able to increase the phosphorylation of the LCK Y394-positive regulatory tyrosine; and (iii) block the tyrosine phosphorylation of a number of cellular proteins mediated by the expression of a Y505F constitutively active LCK mutant. Furthermore, these inhibitory effects have been shown to be mediated by the TSAd carboxyl region, specifically, the same phosphorylated tyrosine residues and proline-rich stretch that mediate interaction with the LCK SH2 and SH3 domains, respectively (29–31). An ability of overexpressed TSAd to inhibit LCK activation would be consistent with those studies that have reported an inhibitory effect of TSAd overexpression upon IL-2 promoter activation. Again, however, the physiological relevance of these overexpression studies is questionable, as in T cells from TSAd-deficient mice, activation of LCK in response to TCR triggering is impaired not augmented (see subsequent paragraphs). Also, as LCK kinase activity has not been directly examined in these overexpression studies, it is not entirely clear that the observed effects are indeed a consequence of reduced LCK catalytic activity *per se* or to some other influence. In this regard, it has been proposed that TSAd may act as an LCK phosphorylation

sink that redirects LCK kinase activity when overexpressed in T cells (31).

Counter to the aforementioned findings, *in vitro*, recombinant TSAd has been shown to activate directly the kinase activity of LCK, as measured by an ability of the kinase to phosphorylate a peptide substrate and itself in *in vitro* kinase assays (17). Although in those instances where overexpressed TSAd has been shown to augment TCR-induced IL-2 promoter activity, there has not been a parallel demonstration of enhanced LCK activity; the finding that recombinant TSAd can activate LCK *in vitro* is consistent with this augmentation as well as the finding that TCR-induced activation of LCK is impaired in TSAd-deficient T cells (17). The molecular mechanisms by which TSAd may activate LCK in T cells are discussed in more detail in the context of the TSAd-deficient mouse (see the following section).

A nuclear function for TSAd

Aside from regulating proximal TCR signal transduction, TSAd may also have a nuclear role in T cells. Indications of a nuclear role were first suggested by the observation that a substantial fraction of both transfected and endogenous TSAd is located in the nucleus of Jurkat T cells (27). This nuclear translocation is mediated by the TSAd SH2 domain, as a form of human TSAd that contains a point mutation in the SH2 domain that abrogates its ability to recognize phosphotyrosine ligands (TSAd R120K) resides exclusively in the cytoplasm. In addition, the TSAd SH2 domain alone traffics to the nucleus when transfected into Jurkat, whereas the same isolated SH2 domain with an R120K point mutation does not. Recently, a form of TSAd that lacks the central part of the carboxyl region that contains a proline-rich stretch plus four tyrosine residues has also been shown to be restricted in expression to the cell cytoplasm (30). This latter finding, therefore, illustrates the complexity of the mechanisms controlling nuclear import of TSAd, when one considers that the entire carboxyl region of TSAd is apparently dispensable for nuclear import as evidenced by the nuclear translocation of the isolated TSAd SH2 domain (27). Nonetheless, available data are consistent with the view that at least a component of the import process involves TSAd SH2 domain recognition of a tyrosine-phosphorylated protein that transports TSAd into the nucleus.

To identify ligands of the TSAd SH2 domain that might be involved in nuclear import, Marti *et al.* (27) performed pull-down experiments of pervanadate-treated Jurkat lysates using a glutathione s-transferase (GST)-TSAd SH2 domain fusion protein. Two main tyrosine-phosphorylated proteins were identified in these experiments of 95–100 and 57 kDa plus minor species in the 35–40 kDa range. However, only the 95–100-kDa protein was found in the T-cell nucleus as well as the cytoplasm, suggestive of a role in nuclear transport. Using microchemical techniques, this ligand was unequivocally identified as p95 valosin-containing protein (VCP) (32). VCP is a highly conserved large hexameric protein that belongs to the adenosine triphosphatase (ATPase) associated with diverse cellular activities (AAA) family (33, 34). It functions in numerous different cellular processes that in part may be explained by its role in the targeting of ubiquitylated proteins to the proteasome for limited or complete proteolytic digestion (35–38). VCP has previously been shown to be required for nuclear import of the NFκB transcription factor in mammalian cells (39). In addition, the yeast homolog of VCP, CDC48, has been shown to be necessary for nuclear import of SPT23 and Mga2 transcription factors into the yeast nucleus (40, 41). In TCR-stimulated T cells, VCP is phosphorylated upon a tyrosine residue that is the penultimate tyrosine residue contained within each hexameric subunit and in yeast phosphorylation of this same residue in CDC48 has been shown to stimulate nuclear mobilization of CDC48 (42–44). Interestingly, TSAd SH2 domain binding to VCP is dependent upon phosphorylation of this same tyrosine residue, although biochemical analysis indicates that TSAd does not bind directly to this residue (32). Most importantly, in yeast cells, nuclear translocation of transfected TSAd was shown to be dependent upon VCP/CDC48 as in yeast that lack functional CDC48, nuclear translocation was not observed (32). Together, these findings suggest a model in which TCR engagement in T cells leads to phosphorylation of VCP upon a penultimate tyrosine residue, which allows TSAd SH2 domain recognition of this chaperone. In addition, the same phosphorylation promotes nuclear import of VCP and, by association, TSAd. However, whether VCP is essential for TSAd nuclear mobilization in T cells has yet to be established.

The function of TSAd in the nucleus is largely unknown, but it is of interest that when fused to the DNA-binding domain (DBD) of the GAL4 transcription factor, TSAd is able to drive transcription from a GAL4-operator luciferase reporter in T-cell lines (27). Furthermore, this transcription trans-activating ability depends upon an intact TSAd SH2 domain, as a GAL4-DBD-TSAd (R120K) mutant is unable to activate transcription from the same reporter despite that this fusion is translocated to the nucleus owing to the presence of a strong nuclear localization sequence in the GAL4-DBD component. Therefore, in this system, TSAd appears to activate transcription indirectly through interaction with a ligand of the SH2 domain that may or may not be VCP. It seems improbable that

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TSAd itself would directly bind to DNA. Rather, if TSAd were naturally to function in gene transcription in the nucleus, then this would likely involve physical interaction with a *bona fide* DNA-binding protein. If so, TSAd might be considered as a transcription adapter protein that facilitates complex formation between proteins with DNA-binding ability on the one hand and proteins with transcription-activating ability on the other.

TSAd-deficient mice

Rajagopal *et al.* (16) generated TSAd-deficient mice by homologous recombination in embryonic stem (ES) cells. Analysis of young mice showed normal numbers of T cells and normal ratios of different T-cell subsets in thymus, lymph node, and spleen, leading to the view that TSAd is not required for T-cell development or peripheral T-cell homeostasis. However, functionally, splenic TSAd-deficient T cells were shown to proliferate less and synthesize reduced amounts of IL-2 and interferon-γ (IFN-γ) in response to CD3 and CD3 plus CD28 mAb stimulation *in vitro*. These last findings, therefore, clearly illustrate the role of TSAd as a positive regulator of TCR signal transduction.

Subsequent analysis of these mice confirmed and extended these observations. Through crossing these mice to HY TCR and AND TCR transgenic mouse strains, TSAd was shown to be dispensable for the thymic development of major histocompatibility complex (MHC) class I- and class II-restricted T cells, respectively (45). Both positive and negative selection proceeded normally in the absence of TSAd. Gene chip microarray experiments of purified splenic $CD4^+$ T cells from wild-type and TSAd-deficient mice stimulated with CD3 and CD28 mAb confirmed a substantial reduction in the ability of TSAd-deficient T cells to synthesize IL-2 and IFN-γ (46). In addition, however, IL-4 mRNA was also found to be reduced in stimulated TSAd-deficient T cells. At the protein level, HY TCR transgenic and AND TCR transgenic TSAd-deficient peripheral T cells were found to secrete reduced amounts of IL-2 and IFN-γ in response to stimulation with cognate peptide–MHC complexes *in vitro* (authors' unpublished data).

Role of TSAd as a regulator of LCK in primary T cells

Consistent with impaired cytokine synthesis, several different TCR-induced cytoplasmic signaling events are dampened in TSAd-deficient T cells (17). Thus, there is reduced activation of the Ras small GTP-binding protein and downstream activation of mitogen-activated protein kinases (MAPKs) that converge upon AP-1. In addition, calcium mobilization from intracellular stores is reduced in TSAd-deficient T cells. These defects can be traced backwards to reduced interaction of PLC-γ and the adapter protein Grb-2 with LAT, reduced tyrosine phosphorylation of LAT, reduced activation of ZAP70 and phosphorylation of the TCR-ζ chain and, ultimately, impaired activation of LCK (17). This impaired activation of LCK is apparent both as reduced phosphorylation of the Y394-positive regulatory tyrosine in the LCK kinase domain as well as reduced kinase activity *per se* as measured in *in vitro* kinase assays. In summary, therefore, these studies show that TSAd has an essential role in the activation of LCK in peripheral T cells and that this function, at least in part, accounts for the role of TSAd as a regulator of T-cell function. Interestingly, this role for TSAd appears to be restricted to peripheral T cells, as in developing TSAd-deficient T cells, activation of LCK is not impaired. Why activation of LCK in thymocytes does not require TSAd is unknown at present.

It seems likely that the mechanism by which TSAd promotes the activation of LCK in peripheral T cells involves interruption of intramolecular inhibitory interactions of the kinase. In this regard, LCK SH2 domain recognition of phosphorylated LCK Y505 is thought to promote an interaction of the LCK SH3 domain with an LCK proline-rich stretch contained in a linker region between the SH2 and kinase domains, the effect of which is to close the kinase into an inactive conformation (11, 28, 47, 48). Therefore, through binding to the LCK SH2 and SH3 domains, TSAd would drive LCK into an open conformation, from which point it has the potential to become activated by phosphorylation upon Y394. Analysis of the structural requirements of TSAd in terms of its ability to activate LCK kinase activity *in vitro* is consistent with this view. Thus, mutation of the phosphorylated TSAd carboxyl region tyrosine residues or of the central proline-rich stretch abrogates an ability of full-length TSAd to activate LCK in this assay (17).

In response to TCR and CD4/CD8 coreceptor engagement, it might be envisaged that activation of LCK initially proceeds in a TSAd-independent manner. This process could occur as a result of cross-phosphorylation on Y394 of those LCK molecules that pre-exist in an open conformation. This activation would result in an increase in the kinase activity of those LCK molecules that would be sufficient to mediate phosphorylation of TSAd on carboxyl region tyrosine residues. Consequently, TSAd would compete with LCK Y505 for binding to the LCK SH2 domain and thereby drive additional LCK molecules into an open primed conformation. In addition, at this point, interaction of the proline-rich region of TSAd with the LCK SH3 domain might promote or stabilize adoption of

the open conformation in a manner that would not have been possible prior to TSAd tyrosine phosphorylation either because tyrosine phosphorylation is required to unmask the proline-rich region and/or that interaction of the proline-rich region with the LCK SH3 domain cannot occur until displacement of the LCK SH2 domain from phosphorylated Y505. Still further stabilization of the open conformation might then result from the action of the transmembrane protein tyrosine phosphatase CD45, which dephosphorylates Y505 of LCK (49). Once driven into the open conformation, full activation of these LCK molecules would then occur as a result of cross-phosphorylation on Y394.

In the aforegiven model, TSAd can be viewed as participating in a positive feedback loop that recruits additional LCK molecules into cycles of activation, that is, LCK activation results in TSAd phosphorylation, which leads to increased LCK activation, which leads to increased TSAd phosphorylation, and so on. However, recent unpublished data from our laboratory suggest an alternative mechanism through which TSAd may promote LCK activation in T cells. We have determined that the SH2 domain of TSAd binds to a terminal phosphorylated tyrosine residue of the LAT molecule Y235. Indeed, LAT is likely to represent one of the 35–40-kDa tyrosine-phosphorylated proteins identified in GST pull-down experiments using the TSAd SH2 domain (27). Physical interaction of TSAd with LAT has been demonstrated both in Jurkat and in primary mouse lymph node T cells. By virtue of a palmitoylation modification, LAT is found almost exclusively in glycolipid-enriched rich lipid rafts in T cells (50, 51). It is predicted therefore that TSAd would be targeted to lipid rafts in T cells during the course of TCR signaling. Indeed, in Jurkat cells, TSAd translocates to lipid rafts in response to TCR triggering; however, no such translocation of TSAd to lipid rafts is observed in the JCam2.5 LAT-negative variant of Jurkat, thus illustrating the critical role of LAT in TSAd targeting to rafts. The question then is what is the purpose of TSAd translocation to rafts? Might this translocation relate to the ability of TSAd to regulate the activity of LCK? Preliminary information indicates that this is in fact the case, in that TCR-induced physical association of TSAd with LCK is seen to occur only within rafts and is again dependent upon LAT. However, it is thought that the LCK that resides within rafts has become recently activated outside rafts, whereupon it translocates into rafts as a result of activation-induced palmitoylation (52, 53). Furthermore, within rafts, LCK is thought to set forward a series of events that result in its phosphorylation by the C-terminal Src kinase (CSK) upon Y505 that causes a refolding of LCK back into an inactive conformation (2, 54–56). With this information, TSAd might not contribute to the initial activation of LCK outside rafts but instead might function to prevent LCK inactivation within rafts by inhibiting this refolding.

T-cell migration

In addition to TCR signaling, TSAd has been reported to play a positive regulatory role in chemokine receptor signaling in T cells. Following upon their identification of TSAd as a binding partner of Gβ subunits of heterotrimeric G proteins in yeast hybrid screens, Park *et al.* (57) demonstrated that in Jurkat, TSAd physically associates with Gβ in response to stimulation with the chemokines CXCL12 and CCL5. More importantly, short interfering RNA (siRNA)-mediated inhibition of TSAd expression in Jurkat impeded directed migration induced by CXCL12 and CCL5. Conversely, overexpression of TSAd increased Jurkat cell CXCL12- and CCL5-induced migration. How, during the course of chemokine receptor signaling, TSAd links Gβ to the cellular migration response is unclear. However, both CXCL12 and CCL5 were also shown to promote physical interaction of TSAd with LCK and ZAP70 in Jurkat, both of which are known to be necessary for chemokine-induced migration (58–60). Furthermore, inhibition of TSAd expression in Jurkat blocked chemokine-induced activation of ZAP70, thus illustrating an important role for TSAd in ZAP70 activation in this signaling pathway, analogous to the same requirement for TSAd for ZAP70 activation during TCR signal transduction (57). Based upon these findings, it has been proposed that TSAd functions to recruit LCK and ZAP70 to the Gβ signaling complex, leading to the activation of ZAP70 and the triggering of downstream responses such as activation of MAPK and phosphorylation of focal adhesion proteins which drive the chemotactic response.

TSAd and autoimmune disease susceptibility

Although young TSAd-deficient mice appear relatively normal, with increasing age they present with features of lupus-like autoimmune disease that include hypergammaglobulinemia and production of antibodies against self-antigens such as single- and double-stranded DNA, cardiolipin, and self-immunoglobulin (Ig) (46, 61). Frequently, there are signs of glomerulonephritis associated with deposition of IgG complexes in kidney glomeruli. In addition, leukocytic infiltration into non-lymphoid organs is observed. In addition to developing spontaneous lupus-like disease in late age, young TSAd-deficient mice show high susceptibility to experimental pristane-induced lupus that manifests as the development of much higher serum autoantibody titers and more severe

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glomerulonephritis compared with controls (46). In the spontaneous disease, disease development is associated with increased numbers of peripheral T cells that show an activated phenotype. This observation is consistent with the view that the disease is of T-cell origin.

Exactly how T-cell immunological tolerance breaks down in TSAd-deficient mice is uncertain. As noted before, T-cell-negative selection proceeds normally in the absence of TSAd. In addition, natural $CD4^{+}CD25^{+}$ T-regulatory cells (Tregs) that normally guard against the development of autoimmune disease are found in normal numbers in the thymus and peripheral lymphoid organs of TSAd-deficient mice, and no decreases in natural Treg number are apparent with age (authors' unpublished data). We have also determined that adaptive Tregs are generated normally in TSAd-deficient mice in response to transforming growth factor-β (TGFβ) stimulation. Moreover, TSAd-deficient Tregs show an increased and not decreased capacity to suppress T-cell proliferative responses *in vitro*. Thus far, the only abnormality that has been observed in TSAd-deficient mice that might account for autoimmune disease development is a reduced susceptibility of T cells to apoptotic death *in vivo* (45, 46, 61). An impaired T-cell death response in TSAd-deficient mice was revealed following immunization of mice with staphylococcal superantigen. After an initial normal T-cell clonal expansion, responding cells failed to undergo apoptotic death to the same extent as that seen in controls. In other mouse models where there is an impaired death response of peripheral T cells, this is usually associated with the development of lupus-like disease (61, 62). However, the molecular basis for the impaired T-cell death response in TSAd-deficient mice is uncertain. Gene chip analyses and follow-up protein expression analyses of activated TSAd-deficient and wildtype T cells do not reveal any obvious increases in the expression of anti-apoptotic genes or decreased expression of pro-apoptotic genes in TSAd-deficient T cells that might account for the resistance to death. One exception is IL-2, which is known to promote T-cell death *in vivo*, including superantigen-induced death (63–65). TSAd-deficient T cells synthesize severalfold less IL-2 protein than control T cells following superantigen stimulation *in vivo* (46). Therefore, impaired synthesis of IL-2 may be a significant factor that promotes the development of lupus-like disease in TSAd-deficient mice. However, a caveat to this interpretation is the normal frequency and function of Treg in TSAd-deficient mice. IL-2 is required for maintenance of Treg number and Treg function in mice (66–71). Thus, unless one envisages that IL-2 levels in TSAd-deficient mice are diminished to an extent that they impact upon T-cell death but not Treg number and function, then some other factor must be responsible for impaired T-cell death and the development of autoimmune disease in this model. One other group did not observe an impaired T-cell death response in TSAd-deficient mice following administration of superantigen (20).

Whether mutations or polymorphisms of the TSAd gene contribute or do not contribute to the development of systemic lupus erythematosus has not been examined in humans. However, TSAd polymorphisms do appear to be significantly associated with the autoimmune disease multiple sclerosis in humans (72, 73). The human TSAd promoter contains differing numbers of GA repeats (13–33), and, interestingly, TSAd alleles with fewer numbers of repeats (GA13 or GA16) show significant association with multiple sclerosis in Scandinavian cohorts. Consistent with findings in mice that loss of expression of TSAd predisposes toward autoimmune disease, individuals that are homozygous for TSAd alleles with fewer numbers of GA repeats express reduced amounts of TSAd (72). A similar association of GA13 or GA16 TSAd alleles with disease has also been observed in juvenile rheumatoid arthritis and, recently, chronic inflammatory demyelinating polyradiculoneuropathy (74, 75). However, in the case of the demyelinating disorders (multiple sclerosis included), it is not certain if TSAd allele associations with disease reflect a role for altered TSAd expression in disease etiology or, alternatively, can be explained by a very close linkage of the TSAd gene to the gene for neurotrophic tyrosine kinase receptor 1.

ALX

As with TSAd, a function for ALX in T-cell signal transduction was first suggested by studies that examined the effect of its overexpression in Jurkat T cells. Overexpression was found to inhibit IL-2 promoter activity induced by TCR plus CD28 mAb stimulation or stimulation with staphylococcal superantigen (22). Dissection of this effect upon IL-2 promoter activation revealed that the greatest inhibition could be observed with a promoter that is comprised of the CD28 response element plus an AP-1 binding site (RE/AP1), whereas minimal inhibition was observed using a promoter containing AP-1-binding sites alone. Thus, the overexpressed ALX appears to target signaling pathways that activate the CD28 RE. Further studies examined the structural requirements of ALX with regards its ability to inhibit RE/AP promoter activity in Jurkat (76). Mutation of different tyrosine residues or of proline-rich regions in the ALX sequence, alone or in combination, did not affect inhibitory activity. In contrast, point mutation of the ALX SH2 domain to prevent recognition of phosphotyrosine ligands

abrogated inhibitory activity. Together with the finding that the ALX SH2 alone was sufficient to inhibit promoter activation, these studies pointed to a critical role for the SH2 domain of ALX in this experimental system.

During the course of these studies, it was noticed that like TSAd, a fraction of ALX resides in the nucleus of Jurkat cells (77). In addition, TCR plus CD28 mAb stimulation of Jurkat was shown to result in export of ALX out of the nucleus to the cytoplasm. This observation led to the identification of a conserved LxxxLxxL nuclear export sequence (NES) in the ALX carboxyl region. Mutation of this sequence prevented TCR-/CD28-mediated nuclear export of ALX. Similarly, inhibition of the CRM-1 protein, commonly implicated in nuclear export of proteins, using leptomycin B, blocked nuclear export showing the critical role of the CRM-1-mediated pathway as well as the LxxxLxxL sequence of ALX in this process. The mechanism by which ALX enters the nucleus is less well understood although in transfected epithelial cell lines, deletion of the entire carboxyl region of ALX prevents nuclear import. Also unclear is the purpose of ALX nuclear translocation. However, nuclear export appears to be important for the full inhibitory effect of ALX upon TCR/CD28 or superantigen-induced RE/AP activation in Jurkat. Thus, mutation of the NES in ALX abrogates the inhibitory effect (77).

ALX-deficient mice

To examine the physiological role of ALX in T cells, ALX-deficient mice were generated by homologous recombination in ES cells (78). In these mice, T-cell development was found to proceed normally, and numbers and ratios of different T-cell subsets in peripheral lymphoid organs were unaffected by ALX loss. However, purified peripheral T cells from ALX-deficient mice synthesized increased amounts of IL-2 in response to CD3 plus CD28 mAb stimulation *in vitro* and in response to antigenic stimulation *in vitro* following priming to the same antigen *in vivo*. These findings, therefore, are consistent with the result that overexpressed ALX inhibits TCR-/CD28-induced activation of the IL-2 promoter in Jurkat.

Investigations into the molecular basis for the T-cell hyper-responsive phenotype in ALX-deficient mice yielded some surprising findings in that most canonical TCR signaling pathways seemed to be unaffected including intracellular calcium mobilization and pathways leading to the activation of AP-1 (78). However, one pathway that seemed to be constitutively activated in ALX-deficient T cells was the p38 MAPK signaling pathway. A similar phenotype of increased TCR-induced IL-2 synthesis associated with augmented activation of p38 has been described in knockout mice that lack expression of the LAX transmembrane adapter that is related to LAT (79, 80). This finding suggests that ALX and LAX function in the same negative signaling pathway to regulate IL-2 synthesis in T cells. Along these lines, ALX and LAX have been shown to physically associate with one another when both are transfected into Jurkat and the HEK293 embryonic kidney cell line (78, 81). Mutational analysis has revealed that this association is constitutive and does not require LAX tyrosine residues or the ALX SH2 domain. Cotransfection of ALX and LAX into Jurkat results in LAX tyrosine phosphorylation and this phosphorylation appears to depend upon LCK as LAX is not phosphorylated on tyrosine residues when both proteins are transfected into an LCK-negative variant of Jurkat. Furthermore, the ALX SH2 domain was found to pull-down LCK from Jurkat lysates. These findings have led to a model in which ALX is conceived to bind and juxtapose LCK to LAX leading to LAX phosphorylation with resultant suppression of the p38 pathway (81). This model is interesting but is based thus far only upon studies involving overexpressed proteins in cell lines or upon *in vitro* protein association experiments. Apart from the demonstration of association between cell endogenous proteins, strong support for this model would come from the finding that in ALX-deficient T cells, LAX is not phosphorylated upon tyrosine residues during the course of TCR signal transduction. This finding would illustrate an essential role for ALX in the phosphorylation of this transmembrane adapter. Additionally, although overexpression of LAX in Jurkat causes inhibition of TCR-/CD3-induced RE/AP promoter activity, this inhibition is found not to depend upon LAX tyrosine phosphorylation (81).

Although ALX and TSAd have opposing effects in T cells, Perchonock *et al.* (20) generated ALX/TSAd double deficient mice to study the influence of combined loss of both proteins upon T-cell development and function. Similar to single deficient mice, young double deficient mice showed normal T-cell development and normal numbers and ratios of peripheral T-cell subsets. With regards to IL-2 synthesis, double deficient peripheral T cells behaved similar to ALX single deficient T cells in that they synthesized increased amounts of IL-2 in response to CD3 plus CD28 mAb stimulation. Also, double deficient T cells again showed constitutive activation of p38. Therefore, the effect of loss of ALX expression associated with constitutive p38 activation appears to override the effect of loss of TSAd expression even though in single TSAd-deficient mice this results in impaired TCR-mediated activation of LCK (17). Whether TCR-mediated activation of LCK is normal in double deficient mice has not been determined.

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Unlike TSAd-deficient mice, there is scant evidence that ALX-deficient mice develop spontaneous autoimmune disease, although with increasing age some mice show splenomegaly and increased numbers of T cells with an activated phenotype (78). This observation is in accordance with the finding that superantigen-mediated deletion of peripheral T cells is unaffected in ALX-deficient mice. However, the same group that reported these findings was also unable to demonstrate impaired superantigen-induced T-cell death in TSAd-deficient mice or in ALX/TSAd double deficient mice. Whether ALX-deficient mice show or do not show increased susceptibility to experimentally induced lupus has not been determined.

SH2D4A/SH2D4B

To identify ligands of SH2D4A, we performed GST pull-down experiments from Jurkat lysates using the full-length SH2D4A protein (authors' unpublished data). In these experiments, a major protein band of 80 kDa was identified that upon microchemical analysis was determined to represent a mixture of two SH3 domain-containing adapter proteins, hematopoietic-specific protein-1 (HS1), and cortactin, with the former representing the major component (82, 83). For HS1, interaction with SH2D4A was further demonstrated by coimmunoprecipitation and Western blotting in both Jurkat and primary human T cells. Interaction was constitutive and not modulated in response to TCR engagement. Two other specific ligands of SH2D4A, SH3P7 and SH3P8, were identified in yeast-hybrid screens. Like HS1 and cortactin, these proteins contain SH3 domains (84, 85). Interaction of SH2D4A with these different SH3 domain-containing proteins suggested a potential function for SH2D4A in T-cell signal transduction, possibly through regulation of the actin cytoskeleton. HS1, for example, has been shown to control the accumulation of F-actin in the immunological synapse in T cells (86). Furthermore, T cells from HS1- and SH3P7-deficient mice show reduced proliferation and cytokine secretion in response to TCR stimulation *in vitro* (87, 88). In support of a role for SH2D4A in TCR signal transduction, overexpression in Jurkat was found to block TCR-induced expression of the CD69 early activation marker.

To address definitively if SH2D4A played an essential role in TCR signal transduction, we generated SH2D4A-deficient mice (25). In these mice, T-cell development and peripheral T-cell homeostasis was normal. Furthermore, peripheral T cells in SH2D4A-deficient mice synthesized normal levels of cytokines, proliferated normally, and showed normal death responses upon stimulation with CD3 and CD28 mAb. Additional evidence that SH2D4A is not required for T-cell function came from the finding that SH2D4A-deficient mice were able to clear *Listeria monocytogenes* infection *in vivo* with similar efficiency as wildtype littermate control mice. To address if there was a species difference between mouse and humans with respect to a role for SH2D4A in T cells, we examined the effect of knockdown of SH2D4A expression in primary human T cells using siRNAs (25). However, despite the effectiveness of siRNA in reducing SH2D4A expression, no effect of this reduced expression upon CD3 plus CD28 mAb-induced T-cell cytokine synthesis, proliferation, or survival was apparent. SH2D4A, therefore, appears to be dispensable for T-cell signal transduction. Given the high degree of homology between SH2D4A and SH2D4B, it is possible that SH2D4B substitutes for the function of SH2D4A in SH2D4A-deficient T cells. SH2D4B-deficient mice have not yet been reported, and whether SH2D4B has or does not have a non-redundant role in T cells is unknown. Should a role for SH2D4B in T-cell signal transduction not be revealed in SH2D4B-deficient mice, this would emphasize the importance of generating SH2D4A/SH2D4B double deficient mice to address any potential issue of redundancy.

Other functions of TSAd family adapter proteins

TSAd

TSAd has been identified as an interacting partner of several other types of bait protein in yeast-hybrid screens. These include PLC-γ, phosphatidylinositol-3-kinase, the Grb-2 adapter, Smad proteins, the MEKK2 MAPK kinase (MAP3K), and the vascular endothelial growth factor receptor-2 (VEGFR2) (18, 89–93). In those instances where convincing interaction between cell endogenous proteins in mammalian cells has not been subsequently demonstrated, given that yeast-hybrid screening frequently yields false-positive results, these findings should be taken with caution. This approach is particularly true for interactions involving TSAd in yeast because of the strong intrinsic transcription trans-activating potential of the full-length TSAd protein (27). Clearly, however, for some of these binding partners, such as MEKK2 and VEGFR2, the interaction is meaningful and has been shown to be of physiological relevance.

Big MAPK activation

MEKK2 is a MAP3K in the big MAPK signaling pathway (91). It associates with and activates the MAP2K MEK5, which in turn activates the big MAPK ERK5. In cell lines such as HEK293, activation of MEKK2 is required for the activation of

ERK5 during the course of epidermal growth factor receptor (EGFR) signaling or stimulation of cells with sorbitol, H_2O_2, or expression of a constitutively active form of the c-Src PTK. In response to these stimuli, MEKK2 is phosphorylated on tyrosine residues, and this phosphorylation has been shown to be necessary for an increase in MEKK2 kinase activity and stimulation of the big MAPK response. TSAd was identified as an interacting partner of the amino-terminal regulatory domain of MEKK2 in yeast (91, 92). The region of MEKK2 required for interaction was shown to be contained within residues 228–282 of the amino-terminal domain, although the region of TSAd protein that is involved in binding was not clearly identified. Nonetheless, antisense knockdown of TSAd or inhibition of the association between MEKK2 and TSAd, through expression of the MEKK2 228–282 polypeptide in cells, inhibited MEKK2 tyrosine phosphorylation and the activation of ERK5 (92). These findings show that TSAd interaction with MEKK2 is required for MEKK2 tyrosine phosphorylation and activation of ERK5 stimulated by the aforementioned agents. For all of the stimuli, MEKK2 tyrosine phosphorylation was shown to be mediated by Src family PTKs (92). One way to explain these findings is that TSAd functions as an adapter protein in these responses that juxtaposes Src family PTKs to MEKK2, permitting effective MEKK2 phosphorylation. Indeed, TSAd becomes phosphorylated in HEK293 cells in response to these agents, which would allow association with the LCK SH2 domain (92). Furthermore, based upon findings in T cells, there is a strong likelihood that TSAd would promote the activation of Src family PTKs, which would constitute an additional basis for the requirement of TSAd in MEKK2 phosphorylation. In T cells themselves, the significance of TSAd interaction with MEKK2 has not been explored. However, it is of note that upon stimulation of the murine D10 T-cell line with antigen-presenting cells (APCs) bearing specific antigenic peptide–MHC complexes, TSAd is seen to redistribute with MEKK2 to the region of close T cell–APC apposition (91).

Angiogenesis

VEGFR2 is a receptor PTK that upon recognition of its ligand, VEGF-A, promotes blood vessel endothelial cell growth, survival, and migration (94). VEGFR2 is essential for blood vessel vasculogenesis and angiogenesis (the growth of new blood vessels from existing blood vessels). During the course of VEGFR2 signal transduction, several cytoplasmic region tyrosine residues become phosphorylated, including residue Y951 located in the kinase insert domain. In yeast-hybrid screens, TSAd was identified to interact directly with this tyrosine residue (89). In addition, in human umbilical vein endothelial cells and porcine aortic endothelial cells, VEGF-A stimulation was shown to induce association between cell endogenous VEGFR2 and TSAd as well as TSAd tyrosine phosphorylation and interaction of TSAd with c-Src (18, 89). Importantly, TSAd association with phosphorylated Y951 of VEGFR2 was shown to be required for VEGFR2-induced actin stress fiber formation and migration of endothelial cells *in vitro*. Thus, an Y951F mutation of the VEGFR2 cytoplasmic domain, a phosphorylated VEGFR2 peptide spanning Y951, and TSAd knockdown all resulted in inhibition of these VEGFR2-induced responses (18). Moreover, in TSAd-deficient mice, growth of injected fibrosarcoma cells was found to be significantly impaired compared with growth of the same tumor in control mice and this correlated with a reduced blood vessel angiogenesis response toward the tumor in the TSAd-deficient mice (18). These findings demonstrate an important role for TSAd in blood vessel angiogenesis through association with VEGFR2. The precise role played by TSAd in this pathway remains uncertain. However, activation of c-Src is likely a key event in VEGFR2-induced endothelial migration (95). Therefore, an ability of TSAd to promote the activation of Src-family PTK, as demonstrated in T cells, may again provide a basis for its involvement.

PDGFR signaling

One other signaling pathway in which TSAd has been implicated is that initiated by another receptor PTK, the platelet-derived growth factor receptor (PDGFR). Based upon the identification of TSAd transcripts and protein in lung tissue, Park *et al.* (19) asked if TSAd was involved in the PDGFR signaling pathway in lung epithelial cell lines. Stimulation of these cell lines with PDGF resulted in TSAd tyrosine phosphorylation and physical association with the PDGFR and Grb-2. Interaction of TSAd with the PDGFR was shown to be mediated by TSAd SH2 domain recognition of a tyrosine residue contained in the kinase insert domain of the PDGFR, whereas Grb-2 association with TSAd was shown to involve Grb-2 SH2 domain recognition of TSAd carboxyl region tyrosine residue Y292. The significance of these interactions for PDGFR signaling is unclear. However, that they may potentiate PDGFR signaling is suggested by the observations that overexpression of wildtype TSAd but not an Y292F TSAd mutant in a lung epithelial cell line augments PDGFR-mediated activation of AP-1. Conversely, expression of the TSAd SH2 domain alone in these cells inhibits PDGF-mediated AP-1 activation (19).

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ALX

One other function that has been attributed to ALX is regulation of B-cell apoptosis. Principally, this contention has been based upon the finding that overexpression of ALX in the WEHI-231 B-lymphoma cell line inhibits antigen receptor-induced death associated with increased activation of the JNK MAPK and inhibition of mitochondrial depolarization (23). Furthermore, stimuli that promote B-cell death such as antigen receptor engagement and IL-21 treatment significantly reduce the expression of ALX, whereas stimuli that promote B-cell survival such as CD40, BAFF receptor, TLR4, and TLR9 engagement cause an upregulation of ALX expression (23, 24). These findings have led to the contention that ALX functions as an anti-apoptotic protein in B cells. Despite these findings, however, in ALX-deficient mice, B cells develop normally, show normal proliferative and survival responses to a panel of different stimuli *in vitro*, and function normally in the generation of a T-cell-dependent antibody response to nominal antigen *in vivo* (78). In addition, the lack of a B-cell phenotype in these mice cannot be explained by an overlapping function with TSAd, as B cells in ALX/TSAd double deficient mice also function normally (20). Therefore, unless SH2D4A or SH2D4B is able to substitute for the function of ALX and TSAd in B cells, these findings force the conclusion that B-cell apoptosis is in fact not regulated by ALX.

Conclusions

In the last several years, considerable advances have been made with respect to our understanding of the function of TSAd and ALX in T cells and other cell types, aided principally by the development and characterization of knockout mouse models of these adapter proteins. TSAd has been found to function as an important positive regulator of TCR-induced T-cell cytokine synthesis, chemokine-induced T-cell migration, VEGFR2-induced endothelial cell migration, and blood vessel angiogenesis, and EGFR-induced activation of the big MAPK pathway. In contrast, it has become apparent that ALX functions as a negative regulator of TCR-induced cytokine synthesis in T cells. One unifying theme that has emerged from these studies that may explain these different roles is an ability of these adapter proteins to regulate the activation and/or localization of Src family PTK to substrates at the outset of signaling cascades. Thus, in T cells, TSAd acts to activate LCK and/or potentiate its activation in lipid rafts, whereas ALX may act to juxtapose LCK to LAX and thereby participate in a negative feedback pathway that controls the TCR signaling response. TSAd in T cells may play a similar LCK-activating role during the course of chemokine-induced migration. In the VEGFR2-induced endothelial cell migration response, TSAd is likely to be an important factor controlling the activation of Src and in EGFR-induced activation of the big MAPK pathway, TSAd may serve to both activate Src and localize this PTK to MEKK2.

A number of questions regarding the role of this family of adapters have yet to be answered. First and foremost, the function of SH2D4A has remained elusive, and the role of SH2D4B has yet to be studied. Potentially, the lack of phenotype of the SH2D4A-deficient mouse can be explained by an overlapping function with SH2D4B and/or TSAd or ALX, and this possibility needs to be addressed. Also unclear is the purpose of nuclear translocation of TSAd and ALX and whether this relates to a transcription function of these adapter proteins in T cells. Finally, the precise mechanisms by which loss of TSAd expression predisposes toward the development of autoimmune disease in mice has not yet been firmly established and warrants further investigation. Given the reported association of TSAd promoter polymorphisms with multiple sclerosis, demyelinating polyradiculoneuropathy, and juvenile rheumatoid arthritis, delineating these mechanisms are of potential direct relevance to our understanding of the etiology of these human autoimmune diseases.

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