

## T cell-specific adapter protein

with

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### What is T cell-specific adapter protein?

**Philip D. King:** T cell-specific adapter (TSAd) protein is an intracellular signaling adapter molecule that is expressed predominantly in T lymphocytes (1,2). It is less well studied than other adapter proteins in lymphocytes, e.g. SLP-76, Grb-2, LAT, but no less important (3). As an adapter, TSAd lacks catalytic activity but does contain a Src-homology-2 (SH2) domain with the potential to recognize phosphorylated tyrosine residues in other signaling proteins. In addition, carboxyl to the SH2 domain is a proline-rich stretch and several

tyrosine residues present in consensus phosphorylation motifs that can be recognized by SH3 and SH2 domains of other signaling proteins respectively.

Within the T cell lineage, TSAd is expressed at constitutively high levels in CD4<sup>+</sup>CD8<sup>+</sup> double-positive thymocytes and at somewhat lower levels in mature CD4<sup>+</sup> and CD8<sup>+</sup> single-positive T cells in the periphery (4,5). Expression of TSAd is increased in mature T cells in response to T cell antigen receptor (TCR) engagement.

### What do we know about the function of TSAd in T cells?

**Philip D. King:** We are beginning to learn quite a lot about the function of TSAd in T cells, mostly as a result of studies of knockout mice that lack expression of the TSAd protein (4). T cell development is apparently normal in TSAd-deficient mice despite the high level of expression in thymocytes. In addition, in young mice, numbers and ratios of different T cell subsets in the periphery are normal. However, peripheral T cells from TSAd-deficient mice produce reduced quantities of key cytokines such as IL-2, IFN- $\gamma$  and IL-4 in response to TCR stimulation *in vitro* and *in vivo* (4,6). Therefore, TSAd appears to play an important role in the TCR signaling pathway leading to the induction of these cytokines.

Recently, a major route through which TSAd controls T cell cytokine synthesis has been elucidated (7). Spe-

cifically, TSAd is required for normal TCR-induced activation of the Src-family protein tyrosine kinase (PTK), LCK, at the outset of the TCR signaling cascade. Consequently, downstream signaling events such as ZAP-70 PTK activation, LAT phosphorylation, activation of PLC- $\gamma$ 1, intracellular calcium mobilization and activation of the Ras-ERK signaling pathway are blocked. Peculiarly, TSAd only appears to perform this role in mature T cells, not thymocytes, consistent with the lack of a thymic phenotype in TSAd-deficient mice.

The mechanism by which TSAd contributes to the activation of LCK in mature T cells is interesting. Available evidence indicates that at the outset of TCR signal transduction LCK phosphorylates tyrosine residues in the TSAd carboxyl-region. These phosphorylated tyrosine residues are then bound by the SH2 domain

of LCK and, in addition, the proline-rich stretch of the TSAd carboxyl-region is bound by the LCK SH3 domain. As a result, previously inactive LCK molecules are switched into an active conformation leading to increased TSAd tyrosine phosphorylation and interaction with LCK and so on. In other words, TSAd is envisaged to function as a central player in a positive-feedback mechanism of LCK activation.

Apart from functioning in the cytoplasm, TSAd may also act in the nucleus to control T cell cytokine synthesis (8). In this regard, a substan-

tial fraction of cellular TSAd is found within the nucleus and is transported to that site by an active mechanism that involves TSAd SH2 domain recognition of a phosphorylated nuclear-importing ligand. Recent work implicates Valosin-containing protein (VCP) as the nuclear importing chaperone (9). The exact function of TSAd in the nucleus is uncertain although a direct role in transcription is suggested by the finding that TSAd can behave as a potent transcription activator in reporter assays in a manner that again depends upon the SH2 domain.

### Are there pathological consequences of TSAd-deficiency?

**Philip D. King:** Yes. As TSAd-deficient mice age, a percentage of them develop features of lupus-like autoimmune disease including hypergammaglobulinemia, production of autoantibodies against self targets such as DNA and deposition of immune complexes in kidneys leading to glomerulonephritis (6,10). Furthermore, young TSAd-deficient mice uniformly show increased susceptibility to

experimentally-induced lupus-like disease. Also, in humans, TSAd promoter poly-morphic variants which drive lower levels of TSAd expression in T cells are significantly associated with multiple sclerosis and juvenile rheumatoid arthritis in examined cohorts (11,12). This implies that in humans, as in mice, reduced TSAd expression promotes the development of autoimmune disease.

### How does TSAd regulate autoimmune disease development?

**Philip D. King:** With increasing age TSAd-deficient mice accumulate large numbers of activated T cells with a memory phenotype pointing to T cell dysregulation as a cause of autoimmunity (6). The dysregulation does not appear to be a consequence of impaired central tolerance. Thus, thymic positive and negative selection processes are intact in TSAd-deficient mice (13). In addition, T regulatory cells are present in normal numbers in the thymus and peripheral lymphoid organs

and are fully-functional as inhibitors of effector T cell proliferation. Thus far, the only defect that we have observed in TSAd-deficient T cells that could account for autoimmunity is impaired antigen-induced death in the periphery. The molecular basis for this resistance to death is unclear at present. Impaired synthesis of IL-2 may be one factor since IL-2 has the ability to promote T cell death *in vivo* (14-16). Clearly, this is an area that warrants further investigation.

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