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Role of the LAT Adaptor in T-Cell Development and T_h2 Differentiation

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Abstract

LAT (linker for activation of T cells) is an integral membrane adaptor protein that constitutes in T cells a major substrate of the ZAP-70 protein tyrosine kinase. LAT coordinates the assembly of a multiprotein signaling complex through phosphotyrosine-based motifs present within its intracytoplasmic segment. The resulting "LAT signalosome" links the TCR to the main intracellular signalling pathways that regulate T-cell development and T-cell function. Early studies using transformed T-cell lines suggested that LAT acts primarily as a positive regulator of T-cell receptor (TCR) signalling. The partial or complete inhibition of T-cell development observed in several mouse lines harboring mutant forms of LAT was congruent with that view. More recently, LAT "knock-ins" harboring point mutations in the four COOH-terminal tyrosine residues, were found to develop lymphoproliferative disorders involving polyclonal T cells that produced high amounts of T helper-type 2 (T_h2) cytokines. This unexpected finding revealed that LAT also constitutes a negative regulator of TCR signalling and T-cell homeostasis. Although LAT is also expressed in mast cells, natural killer cells, megakaryocytes, platelets, and early B cells, the present review specifically illustrates the role LAT plays in the development and function of mouse T cells. As discussed, the available data underscore that a novel immunopathology proper to defective LAT signalosome is taking shape.

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1. Introduction

T cells can be divided into two lineages based on the structure of their T-cell antigen receptor (TCR). In the adult mouse, most T cells express a TCR heterodimer consisting of α and β chains, whereas a minor population expresses an alternative TCR isoform made of γ and δ chains. The signal transduction cassettes operated by αβ- and γδ-TCR share many functional components. Among them, the transmembrane adaptor molecule LAT (linker for activation of T cells) is essential in that it coordinates the assembly of a multiprotein signaling complex through phosphotyrosine-based motifs present within its intracytoplasmic segment. The resulting "LAT signalosome" links the TCR to the main intracellular signalling pathways that regulate T-cell development and T-cell function. Early studies using transformed T-cell lines suggested that LAT acts primarily as a positive regulator of TCR signalling. The partial or complete inhibition of T-cell development observed in several mouse lines harboring mutant forms of LAT was congruent with that view. More recently, two distinct LAT "knock-ins" were found to develop lymphoproliferative disorders involving polyclonal T cells that produced high amounts of T helper-type 2 (T_h2) cytokines. This unexpected finding revealed that LAT also constitutes a negative regulator of TCR signalling and T-cell homeostasis. Although LAT is also expressed in mast cells, natural killer cells, megakaryocytes, platelets, and early B cells, the present chapter will be limited to illustrate the role LAT plays in the development and the function of mouse T cells. As discussed, the available data underscore the existence of an immunopathology proper to defective LAT signalosome.

2. The LAT Signalosome

LAT was identified in 1998 as a 36- to 38-kDa integral membrane adaptor protein that constitutes in T cells a major substrate of the ZAP-70 protein tyrosine kinase (PTK) (Zhang *et al.*, 1998). Adaptor proteins lack both enzymatic and transcriptional activities and act as molecular scaffolds through which multiprotein signaling complexes are transiently assembled via phosphotyrosine-based motifs and/or modular protein-protein interaction domains (e.g., Src homology 2 (SH2)-, Src homology 3 (SH3)-, pleckstrin homology (PH)-domains) (Jordan *et al.*, 2003). Adaptor proteins can be divided into transmembrane adaptor proteins (TRAPs) and cytoplasmic adaptor proteins (Kliche *et al.*, 2004). A subset of TRAPs that includes LAT possesses a juxtamembrane CXXC palmitoylation motif (where C denotes cysteine and X denotes any amino acid). Palmitoylation stabilizes the association of LAT with the plasma membrane and targets it to glycosphingolipid-enriched microdomains (GEMs or lipid

rafts). The association of LAT with lipid rafts does not appear, however, essential for its function during T-cell development and T-cell activation (Zhu *et al*., 2005).

The essential role LAT plays in T-cell signalling was first deduced from the analysis of LAT-deficient variants of the Jurkat T-cell line (Finco *et al*., 1998; Zhang *et al*., 1999a). Subsequent biochemical studies helped define the binding partners of phosphorylated LAT molecules and showed that in T cells most of the signalling activity of LAT is funnelled through the four COOH-terminal tyrosine residues found at positions 136, 175, 195, and 235 of the mouse LAT sequence (Figs. 1 and 2) (Lin and Weiss, 2001; Paz *et al*., 2001; Zhang *et al*., 2000; Zhu *et al*., 2003). After TCR-induced phosphorylation, these four tyrosines manifest some specialization in the SH2-domain-containing proteins they recruit. For instance, mutation of tyrosine (Y) 136 primarily eliminates binding of phospholipase C-γ1 (PLC-γ1), whereas the simultaneous mutation of Y175 and Y195, or of Y175, Y195, and Y235 results in loss of binding of the Gads and Grb2/Grap adaptors, respectively (Lin and Weiss, 2001; Paz *et al*., 2001; Zhang *et al*., 2000; Zhu *et al*., 2003). Grb2 comprises a central SH2 domain flanked by two SH3 domains that are constitutively associated with a variety of signalling proteins, including Sos and Cbl. The Grb2-like adaptor Grap is specifically expressed in lymphocytes. Gads resembles Grb2/Grap and contains an additional proline-rich region between its SH2- and

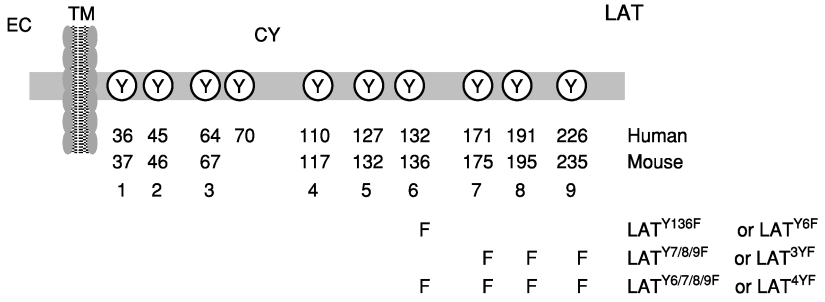

Figure 1 Schematic representation of the mouse and human LAT molecules. The extracellular (EC), transmembrane (TM), and cytoplasmic (CY) segments are indicated together with the tyrosine (Y) residues found within the cytoplasmic region. Human LAT contains 10 tyrosines of which nine are conserved in mouse LAT. Of these, only the five carboxy-terminal tyrosines appear to be phosphorylated upon TCR engagement (Zhu *et al*., 2003). The tyrosines conserved in mouse and human LAT have been numbered 1 to 9 by beginning at the membrane proximal tyrosine. Mutant LAT molecules where the three or the four carboxy-terminal tyrosines were mutated to phenylalanine have been denoted $LAT^{Y7/8/9F}$ (or LAT^{3YF}) and $LAT^{Y6/7/8/9F}$ (or LAT^{4YF}), respectively. Molecules with a mutation that replaced tyrosine 136 with a phenylalanine have been denoted LAT^{Y136F} (or LAT^{Y6F}).

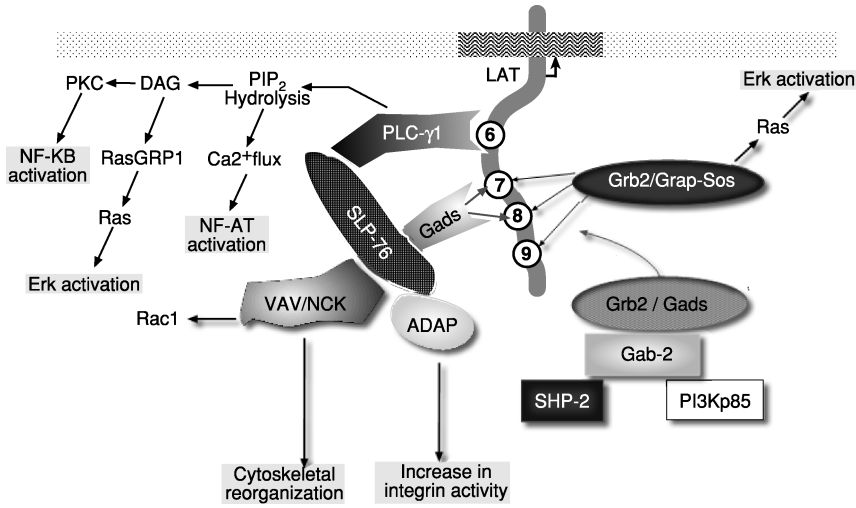

Figure 2 A model of the signalling complexes assembled through LAT in T cells. Raft-associated LAT molecules accumulate in the vicinity of activated TCR and undergo protein tyrosine phosphorylation events. These events are initiated by Src-family PTKs (Lck and Fyn), and proceed through the Syk-family PTK ZAP-70. Once bound to phosphorylated LAT via Gads, SLP-76 is phosphorylated by ZAP-70 and provides a binding site for the SH2-domain of the Tec-family PTK Itk. LAT-bound PLC-γ1 becomes activated following phosphorylation by both ZAP-70 and Itk. The activation of PLC-γ1 leads to the generation of diacylglycerol (DAG) and inositol triphosphate (IP_3). While IP_3 triggers Ca^{2+} fluxes, DAG activates protein kinase C (PCK) and the nucleotide exchange factor RasGRP1, an activator of Ras in T cells. An independent pathway involving the recruitment of Sos trough Grb2 may also connect LAT to the Ras pathway. Phosphorylated SLP-76 also interacts with the cytosolic adaptor protein Nck and with the nucleotide exchange factor Vav. This ternary complex activates the GTPase Rac1, and induces cytoskeletal reorganization. In addition, phosphorylated SLP-76 interacts with the serine-threonine kinase HPK-1, and the adhesion and degranulation promoting adaptor protein (ADAP), thereby altering the function of integrins. LAT is also capable of interacting through Grb2/Gads with the adaptor protein Gab2 and the tyrosine phosphatase SHP-2. The juxtamembrane CXXC motif, which becomes palmitoylated and targets LAT to lipid rafts, is shown by a broken arrow.

COOH-terminal SH3-domains. Gads interacts constitutively with the adaptor SLP-76, thereby recruiting it to LAT, together with its constellation of associated molecules (Vav, Nck, Itk, adhesion and degranulation promoting adaptor protein (ADAP)). SLP-76 contributes to PLC-γ1 activation by stabilizing the LAT-PLC-γ1 association and by bringing the Tec family PTK Itk in the vicinity of its PLC-γ1-substrate (Yablonski *et al*., 2001). In addition to PLC-γ1, another major effector molecule functioning downstream of LAT is the Ras GTPase, whose activation is defective in both *Lat*- and *Slp-76*-deficient T cells. In T cells, the functional coupling between LAT and Ras occurs mainly

through an SLP-76-PLC-γ1-RasGRP1 pathway, and secondarily via a Grb2-Sos axis (Fig. 2).

3. LAT: An Essential Component of the Pre-TCR

Genetic studies have defined two consecutive developmental checkpoints at which T cells progressing along the αβ-lineage undergo programmed cell death if they fail to productively rearrange their TCR genes or express TCR αβ heterodimers with inappropriate specificities (Malissen *et al*., 1999; Von Boehmer *et al*., 2003). Transition through the earliest checkpoint requires the operation of a molecular sensor known as the pre-TCR complex. Once successfully assembled, this multimolecular complex triggers the transition from the double-negative (DN) $CD4^-$ $CD8^-$ stage to the double-positive (DP) $CD4^+CD8^+$ stage, and ensures that only DN cells with a productive TCR β gene rearrangement are rescued from cell death and become DP cells. At the DP stage, a second molecular sensor assembles and controls the transition to the single-positive (SP) $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$ stages on the basis of the specificity of TCR αβ heterodimers. As discussed later, the phenotype of mice deficient in LAT ($Lat^{-/-}$), or having a mutation of the three ($Lat^{Y7/8/9F}$) or four ($Lat^{Y6/7/8/9F}$) COOH-terminal tyrosine residues of LAT underscores the positive regulatory role played by LAT during αβ T cell development and shows that LAT is essential for the function of the pre-TCR (Nunez-Cruz *et al*., 2003; Sommers *et al*., 2001; Zhang *et al*., 1999b).

3.1. αβ T-Cell Development in $Lat^{-/-}$, $Lat^{Y7/8/9F}$, and $Lat^{Y6/7/8/9F}$ Mutant Mice

Compared to wild-type thymi, adult thymi from $Lat^{-/-}$, $Lat^{Y7/8/9F}$, and $Lat^{Y6/7/8/9F}$ mice were hypocellular. They showed a complete absence of DP and SP cells, and contained TCR-β gene rearrangements that were as extensive and diverse as those found in wild-type DN cells. Analysis of the DN compartment found in $Lat^{-/-}$, $Lat^{Y7/8/9F}$, and $Lat^{Y6/7/8/9F}$ thymi for the expression of CD44 and CD25 showed a strict developmental blockade at the $CD44^-CD25^+$ to $CD44^-CD25^-$ transition. This phenotype resembles the one found in thymi of mutant mice unable to assemble a functional pre-TCR (Malissen *et al*., 1999; Von Boehmer *et al*., 2003), and indicates that mutation of the three COOH-terminal tyrosines of LAT suffices to prevent pre-TCR function.

$CD25^+CD44^-$ DN thymocytes from $Lat^{-/-}$, $Lat^{Y7/8/9F}$ and $Lat^{Y6/7/8/9F}$ mice express lower levels of pre-TCR complexes than $CD25^+$ $CD44^-$ DN thymocytes from wild-type mice (Nunez-Cruz *et al*., 2003; Sommers *et al*., 2001). This suggests that LAT normally control the steady-state levels of

pre-TCR complexes at the surface of DN cells. Signal transduction through the pre-TCR, and through the clonotype independent complexes (CICs) that are expressed at the surface of wild-type and of *Rag*-deficient $CD25^{+}CD44^{-}$ DN cells, respectively, can be triggered by injection of anti-CD3 monoclonal antibodies (Wiest *et al*., 1995). CICs consist of calnexin and of either CD3-γϵ or CD3-δϵ polypeptide pairs. Antibody-mediated cross-linking of the pre-TCR and CIC complexes found on $Lat^{-/-}$ and $Lat^{Y6/7/8/9F}$ DN cells did not induce their proliferation and maturation to the DP stage (Nunez-Cruz *et al*., 2003; Sommers *et al*., 2001). In contrast, treatment of $Lat^{Y7/8/9F}$ mice with anti-CD3 monoclonal antibodies induced the development of rare DP cells. This suggests that under the supra-physiological stimulation conditions provided by anti-CD3 treatment, $LAT^{Y7/8/9F}$ molecules can still manifest residual signalling potential in DN cells, an attribute exploited by some developing γδ T cells (see Section 4). Consistent with the view that $LAT^{Y7/8/9F}$ molecules fail to recruit the SLP-76 adaptor, treatment of $Slp\text{-}76^{-/-}$ mice with anti-CD3 monoclonal antibodies induced the development of a few DP cells with a magnitude similar to that observed in $Lat^{Y7/8/9F}$ mice (Pivniouk *et al*., 1998).

3.2. αβ T-Cell Development in Lat^{Y136F} Mutant Mice

To address the importance of LAT Y136 *in vivo* and analyze the consequence of selectively eliminating binding of PLC-γ1, knock-in mice with a mutation that replaced Y136 with phenylalanine (Y136F) were independently derived by two groups (Aguado *et al*., 2002; Sommers *et al*., 2002). Thymi from mice homozygous for this mutation, Lat^{Y136F}, contained approximately tenfold fewer cells than wild-type thymi and showed reduced numbers of DP and SP thymocytes. Analysis of the DN compartment found in Lat^{Y136F} thymi further demonstrated that the Lat^{Y136F} mutation constitutes a hypomorphic (partial loss of function) mutation of the pre-TCR checkpoint. After reaching a peak in mutant newborn mice, DP cells decreased and were almost undetectable in mutant mice older than 7 weeks. Coincident with this progressive DP erosion, a population of CD4 T cells started to dominate the thymus. The phenotype of these CD4 T cells ($CD44^{high}$, $CD62L^{low}$, $CD69^{+}$, and $CD24^{-}$) was distinct from that expected for genuine CD4 SP thymocytes. These CD4 T cells corresponded to abnormal CD4 T cells that, after expanding in the periphery of Lat^{Y136F} mice, infiltrated the thymus among other organs (see Section 5). The IL-5 and IL-13 cytokines they produced in the thymic parenchyma were responsible for tissue-fibrosis and tissue-eosinophilia, and resulted in the subsequent erosion of the DP cell compartment. Consistent with that view, when the development of the infiltrating CD4 T cells was prevented by breeding the Lat^{Y136F} mutation on a genetic background deprived of MHC class II molecules (see later

discussion), the small complement of DP thymocytes characteristics of young Lat^{Y136F} mice remained stable over time (Aguado *et al*., 2002).

Prior to their infiltration by peripheral CD4 T cells, Lat^{Y136F} thymi contain very small numbers of CD4 and CD8 SP thymocytes, suggesting that the Lat^{Y136F} mutation also affects the DP to SP transition (Aguado *et al*., 2002). The absence of CD8 and CD4 T cells in Lat^{Y136F} mice rendered deficient for both MHC class I and MHC class II molecules indicates that the development of these rare SP cells requires a selective process involving MHC class I and MHC class II, respectively (Aguado *et al*., 2002). Therefore, the Lat^{Y136F} mutation negatively affects the two checkpoints that punctuate intrathymic αβ T-cell development and globally results in a severe but partial impairment of αβ T-cell development.

3.3. αβ T-Cell Development in $Lat^{-/-}$ Mutant Mice Ectopically Expressing a NTAL/LAB Adaptor

A transmembrane adaptor protein called NTAL (for non–T-cell activation linker (Brdicka *et al*., 2002)), or LAB (for linker of activation of B cells (Janssen *et al*., 2003)) has been recently identified as the product of the *Wbscr5* gene. NTAL is structurally similar to LAT. It possesses a short extracellular domain, a transmembrane region, two palmitoylated membrane proximal cysteine residues, and a long cytoplasmic tail with several tyrosine residues that are conserved between mouse and human. In B cells and mast cells, NTAL is rapidly tyrosine phosphorylated following ligation of immunoreceptors.

Despite a remarkable conservation of the exon-intron organization of the *Ntal* and *Lat* genes and of the NTAL and LAT structural domains, suggesting that these two adaptors originate from the duplication of an ancestral gene (Brdicka *et al*., 2002), important differences exist, however, in the intracytoplasmic partners capable of binding to LAT or to NTAL. Five of the nine NTAL tyrosines are potential binding sites for Grb2. However, none of the nine tyrosines is in a consensus binding-motif for PLC-γ1. As a consequence, NTAL does not bind to PLC-γ, and thus resembles LAT^{Y136F} mutant molecules. Indeed, when ectopically expressed in developing T cells of LAT-deficient mice, NTAL functionally behaved like LAT^{Y136F} mutant molecules (Janssen *et al*., 2004).

4. Role of LAT in γδ T-Cell Development

As described in Section 3, T cells progressing along the αβ-lineage encounter two consecutive developmental checkpoints. Likewise, thymocytes committed to the γδ-lineage encounter a single checkpoint at the penultimate DN stage ($CD44^{-}CD25^{+}$), which counterbalances the stochastic nature of the