El receptor de células T (TCR) reconoce péptidos unidos al complejo mayor de histocompatibilidad (MHC) y transmite esta información a la célula T a través de proteínas adaptadoras. El adaptador LAT (de «Linker for Activation of T cells») es una proteína transmembrana que, una vez fosforilada en sus residuos de tirosina, coordina la unión de muchas proteínas implicadas en señalización intracelular, de modo que promueve la formación de complejos multi-moléculares que regulan la activación y maduración de las células T. Estudios funcionales y estructurales, tanto in vitro como in vivo, han revelado un papel central de LAT como plataforma para la distribución de señales procedentes del TCR y pre-TCR, así como una inesperada función en la regulación del desarrollo y homeostasis de las células T. En esta revisión se discuten algunos de los más recientes avances acerca de las funciones de este adaptador en la maduración y activación de los linfocitos T.

PALABRAS CLAVE: LAT / Proteínas adaptadoras / Transducción de señales / Receptor para el antígeno (TCR) / Desarrollo tímico.

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The role of the adapter LAT in T cell activation and maturation

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PAPEL DEL ADAPTADOR LAT EN LA ACTIVACIÓN Y MADURACIÓN DE CÉLULAS T

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RESUMEN

El receptor de células T (TCR) reconoce péptidos unidos al complejo mayor de histocompatibilidad (MHC) y transmite esta información a la célula T a través de proteínas adaptadoras. El adaptador LAT (de «Linker for Activation of T cells») es una proteína transmembrana que, una vez fosforilada en sus residuos de tirosina, coordina la unión de muchas proteínas implicadas en señalización intracelular, de modo que promueve la formación de complejos multi-moléculares que regulan la activación y maduración de las células T. Estudios funcionales y estructurales, tanto in vitro como in vivo, han revelado un papel central de LAT como plataforma para la distribución de señales procedentes del TCR y pre-TCR, así como una inesperada función en la regulación del desarrollo y homeostasis de las células T. En esta revisión se discuten algunos de los más recientes avances acerca de las funciones de este adaptador en la maduración y activación de los linfocitos T.

PALABRAS CLAVE: LAT / Proteínas adaptadoras / Transducción de señales / Receptor para el antígeno (TCR) / Desarrollo tímico.

ABSTRACT

The T Cell Receptor (TCR) recognizes peptides bound to major histocompatibility complex (MHC) molecules and relays this information to the T cell through adapter proteins. The adapter LAT (Linker for Activation of T cells) is a transmembrane protein that, once phosphorylated in its tyrosine residues, coordinates the binding of many signaling proteins in order to assemble multi-molecular complexes that regulate T cell activation and maturation. Structure/function studies, both in vitro and in vivo, have revealed a central role of LAT as a platform for the distribution of signals coming from the TCR and the pre-TCR, and also an unexpected function in the regulation of T cell development and homeostasis. Thus, in the present review we discuss some of the recent advances on the role of this adaptor in T lymphocyte development and activation.

KEY WORDS: LAT / Adaptor proteins / Signal transduction / T-cell receptor (TCR) / Thymic development.
INTRODUCTION: LAT AS A PLATFORM FOR THE DISTRIBUTION OF INTRACELLULAR SIGNALS

T lymphocytes are immune cells able to recognize foreign peptides presented by Antigen Presenting Cells (APCs) in the groove of appropriate Major Histocompatibility Complex (MHC) molecules. For this purpose, most T cells express at their surface an antigen receptor composed of the TCRα-β chains, and several invariant polypeptides (the CD3-γ, -δ, -ε and -ζ polypeptides) responsible for intracellular signal transduction. Antigen engagement by the TCR leads to Lck activation, which in turn, phosphorylates the tyrosine residues found in the immunoreceptor tyrosine-based activation motifs (ITAM) in the CD3 chains of the TCR(1-3). Phosphorylation of these motifs recruits the ZAP-70 tyrosine kinase to the TCR/CD3 complex in the membrane, leading to its activation by Lck and, consequently, to the phosphorylation of downstream targets. However, the link of ZAP-70’s enzymatic activity to downstream intracellular signaling pathways, such as Phospholipase C-γ1 (PLC-γ1)-calcineurin-NFAT or MAP-kinases pathways was unknown until recently.

The adaptor LAT was identified in 1998 as an integral transmembrane protein of 36-38 kDa(4,5). LAT presents a single region of about 20 hydrophobic amino acids that constitutes the transmembrane segment and is expressed in the membrane of T lymphocytes, NK and mast cells, platelets, and pre-B cells(4,7). As the majority of adaptors, LAT lacks any enzymatic or transcriptional activity. LAT presents in its amino acid sequence nine conserved tyrosine residues that, upon phosphorylation, are potential docking sites for different proteins (Fig. 1). Indeed, the initial studies demonstrated that LAT is tyrosine phosphorylated by Syk or Lck-activated ZAP-70 kinases. Upon tyrosine phosphorylation LAT binds to Grb2, Grap, Gads-SLP-76, PLC-γ1, Vav, Cbl and the regulatory subunit of PI-3K(4,8,9).

Therefore, adaptor proteins binding to LAT can also bring other associated molecules to these LAT nucleated complexes, and the enzymes recruited can themselves be activated in these complexes by tyrosine phosphorylation and find higher concentrations of their substrates in the plasma membrane. Interestingly, LAT associates with the CD8 T cell coreceptor for MHC and is, thus, concentrated in the proximity of the MHC-engaged TCR, offering a
The possible explanation for its recruitment to the engaged TCR/CD3 complex in CD8 T lymphocytes (10). From these preliminary studies it was clear that tyrosine residues of LAT were essential for its functions, since mutation of tyrosines 7 and 8 of LAT (see Fig. 1) totally prevented the binding of Grb2, the p85 subunit of PI3K, and strongly diminished the TCR-induced activation of AP-1 and NF-AT transcription factors. Therefore, LAT may be regarded as a molecular platform distributing signals coming from the TCR/CD3 complex towards three different downstream pathways: 1) binding to Grb2 links the TCR to the MAP kinases pathway; 2) engagement of PLC-γ1 enhances its catalytic activity leading to Ca2+ influx generation and NF-AT activation; 3) binding to SLP-76 couples the TCR to the cytoskeleton polymerization machinery. The functional significance of LAT in intracellular signals coupled to the TCR was demonstrated by the characterization of the Jurkat cell variants J.CaM2(11) and ANJ3(12). Although both cell lines show drastically reduced levels of LAT, the engagement of the TCR in these cells resulted in normal phosphorylation of the TCR ζ chain and of ZAP-70. However, downstream signaling events such as PLC-γ1, Vav and SLP-76 tyrosine-phosphorylation, Erk activation, Ca2+ influx generation, NF-AT activation, or CD69-induced up-regulation were prevented in these cell lines. Importantly, the re-expression of LAT in J.CaM2 cells totally restored all these downstream events triggered upon TCR engagement.

The characterization of these LAT deficient cell lines allowed the study of the role of the different tyrosine residues of LAT in vitro. Samelson and co-workers stably expressed wild type and several tyrosine to phenylalanine LAT mutants in J.CaM2 cells in order to test the role of individual tyrosines or combinations of them in the cytosolic tail of LAT (13). This work established that tyrosines 7, 8 and 9 share the responsibility of binding to the SH2 domain of Grb2 upon their phosphorylation, since only simultaneous mutation of these three distal residues abolished Grb2 binding. In a similar way, the Gads-SLP-76 complex binds to phosphorylated tyrosines 7 and 8(13,14). Mutation of tyrosine 6 of LAT, which is in a PLC-γ1 SH2 domain consensus binding motif (YVLV), abrogates the binding to this enzyme upon TCR engagement, preventing Erk activation, Ca2+ influx generation and activation of NF-AT. Interestingly, although neither of the PLC-γ1 SH2 domains would be predicted to bind to these three sites, simultaneous mutation of tyrosines 7, 8
and 9 strongly diminished binding and activation of this enzyme, suggesting that they contribute to the stabilization of a quaternary complex consisting of LAT, Gads, SLP-76 and PLC-γ1. The main conclusion from this study was that the four distal tyrosine residues of LAT are essential for the association with critical signaling molecules, and thus for TCR signal transduction.

Also using J.CaM2 cells and the same kind of approach, Lin and Weiss expanded the analysis on the role of LAT tyrosines in vitro(15). The work of these authors showed that a mutant form of LAT in which all the tyrosine residues, except 6, 7 and 9, were mutated to phenylalanine was still able to mobilize Ca²⁺. However, full reconstitution of TCR-dependent Erk activation requires also tyrosines 4 and 9. Very interestingly, this article also demonstrated that reconstitution of tyrosine residues in trans is not enough to reconstitute Erk activation upon TCR engagement. This means that, at least, tyrosines 6, 7 and 8 must be on the same molecule of LAT for a proper transduction of intracellular signals (Fig. 2). These data were corroborated by Abo and colleagues using a biochemical approach(6). By means of pull down assays in Jurkat cells, this group showed that binding of a GST-LAT fusion protein to PLC-γ1 relies on tyrosine 6, and that binding and activation of PI3K does not occur without tyrosines 6 and 7.

**IS LAT A RAFT-ASSOCIATED PROTEIN?**

Among the different domains present in the plasma membrane, the sphingolipid-cholesterol rafts, also known as GEMs (glycolipid-enriched membrane domains)(16,17), have been proposed to be involved in membrane trafficking, cell morphogenesis and signal transduction mechanisms(17,18). A variety of signaling molecules are concentrated in raft domains, including Src family kinases, heterotrimeric and Ras-like G proteins, as well as molecules involved in Ca²⁺-influx(17). Rafts can be isolated as low-density, Triton X-100-insoluble membrane complexes that are enriched in sphingolipids, cholesterol and glycosyl-phosphatidylinositol (GPI)-anchored proteins(19). Lipid rafts have also been implicated in critical functions in immune receptor signaling, and after TCR stimulation of T lymphocytes, phosphorylated TCR-γ-chain and cytoplasmic signaling proteins including Grb2 and PLC-γ1 were shown to partition into lipid rafts(20,21).

The post-translational addition of lipids, such as myristylation or palmitoylation, has been shown to target many signaling proteins such as Src family kinases to the membrane rafts(27,22). The human, murine and rat forms of LAT show in their amino acid sequences two conserved cysteine residues in the juxtamembrane region, and such cysteines are frequently sites for palmitoylation of integral membrane proteins (Fig. 1). Indeed, it has been shown that LAT is palmitoylated, and that palmitoylated LAT predominantly localizes in the lipid rafts(23,24). Importantly, palmitoylation of LAT depends on both juxtamembrane cysteines, and mutation of these amino acids prevents the efficient partitioning of LAT into the rafts, as well as its tyrosine phosphorylation upon TCR engagement, preventing the transduction of signals coming from the TCR(23,24).

However, very recently it has been proposed that lipid raft localization of LAT is not essential for its functions(25). Zhang and co-workers expressed a fusion protein containing the cytoplasmic tail of LAT and the transmembrane and extracellular domains of a non-raft transmembrane adaptor protein, linker for activation of X cells (LAX) that lacks any palmitoylatable cysteine(26). Surprisingly, this fusion protein effectively restored Ca²⁺ flux, MAPK and NFAT activation after TCR engagement in LAT-deficient cells, although it did not localize to the lipid rafts. These data are in contradiction with previous results showing the requirement of juxtamembrane cysteines for LAT functions, and suggesting that raft localization of LAT is essential for an appropriate signal transduction. However, although it has been clearly demonstrated that cysteines 26 and 29 are palmitoylated and that palmitoylation is not required for membrane localization, it does not mean that this palmitoylation mobilizes LAT into the rafts. Due to the physical properties of palmitate attached to LAT, it becomes Triton-insoluble, a crucial criteria for lipid rafts. Detergent extraction, which is normally used to identify lipid rafts biochemically, could possibly induce aggregation of structures that do not exist before extraction.

In this sense, a previous work by Harder and Kuhn could support this hypothesis(27). The authors used a novel procedure to isolate membrane subdomains highly enriched in activated TCR complexes, and found that LAT selectively accumulated in the membrane environment of activated TCR in a tyrosine phosphorylation dependent manner. Interestingly, juxtamembrane cysteines were shown mandatory for accumulation in the membrane environment of activated TCR. However, staining with a lipid dye that preferentially partitions into membrane rafts was neither detectably increased nor excluded in the membrane areas where LAT and TCR were detected. It is also interesting the fact that accumulation of LAT in these membrane areas seems to depend in its cytoplasmic domain, since a Lck-LAT chimera anchored to the membrane via the Lck’s amino-terminal membrane region shows the same behavior as that of wild type LAT. In agreement with this model, Bonello et al
have recently demonstrated that LAT forms two distinct cellular pools, one in the plasma membrane and the other one at the intracellular compartments that co-distribute with transferrin and the TCR-ζ chain, and this distribution depends on the cytoplasmic domain of LAT(28).

Therefore, the partitioning of LAT into the membrane rafts continues to be a topic of debate. On one side there is evidence that juxtamembrane cysteines are needed for the signal transduction functions of LAT(12,23,24). However, the data of Zhang and co-workers show that the transmembrane region of LAT (containing both cysteines) could be substituted by the transmembrane region of LAX, preserving its functions without raft partitioning(25). It is possible that LAT does not directly require S-palmitoylation for its interaction with the TCR in the lipid rafts but for an interaction with another raft-associated regulatory protein, and the transmembrane region of LAX could correctly implement these functions. Additional experiments with different transmembrane fragments of raft- and non-raft-proteins would shed some light on this subject.

**LAT AND αβ T CELL DEVELOPMENT**

The development of T lymphocytes takes place in the thymus in a process characterized by sequential steps that can be defined by the expression of cell membrane proteins(29,30). Progenitor cells coming from the bone marrow do not express the CD4 or CD8 co-receptors at their membrane, and are thus known as double negative (DN) CD4–CD8– thymocytes. DN cells express CD3 associated chains and TCR-ζ chain, but are negative for TCRγ-δ and -β chains.

The rearrangement of the TCR-γ and -δ genes results in the expression of TCRγδ, inducing the exit of γδ T lymphocytes from the thymus (Fig. 3). Alternatively, the rearrangement of TCR-β induces its expression at the cell membrane together with a TCRβ surrogate chain called pTα, and constituting, together with CD3 associated chains, the pre-T cell receptor (pre-TCR). The successful rearrangement of TCRβ and the expression of the pre-TCR drive the differentiation of DN cells to the CD4+CD8+ double positive (DP) stage, where rearrangement of TCRα leads the expression of mature TCRαβ. Last, DP thymocytes undergo positive and negative
selection events to become mature CD4+ or CD8+ single positive (SP) cells.

Targeted gene disruption has been widely used to study the functions of several enzymes or adaptor proteins during T cell development. The same has been done for LAT, revealing its essential role for T cell development(29,30). LAT deficient mice show normal B and NK cell compartments, but a total absence of T lymphocytes in the periphery. Adult thymi from LAT deficient mice are smaller and hypocellular, and thymic development is blocked at the DN-CD25-CD44-stage, with a total absence of DP and SP cells. This demonstrates a crucial role for LAT in the transduction of intracellular signals coming from the pre-TCR and allowing DN to DP transition. The phenotype of these mice resembles the one found in mice unable to ensemble a functional pre-TCR(29,30). Interestingly, although NK cells and platelets express LAT, no obvious abnormality of NK or platelet function and development is observed in LAT−/− mice.

In an attempt to clarify the functions of the different tyrosine residues of LAT in vivo, several Knock In animals harbouring individual mutations of these residues have been developed. In agreement with a positive role for LAT in the transduction of signals coming from the pre-TCR, mice having a mutation of the three (LATY7/8/9F) or four (LATY6/7/8/9F) distal tyrosines of LAT have small thymi with a complete absence of DP and SP cells, and thymic development is also blocked at the DN-CD25-CD44-stage(31,32). Similarly to LAT−/− mice, TCR-β rearrangements in LATY7/8/9F or LATY6/7/8/9F mice were as extensive and diverse as those found in wild type mice, although the expression level of the pre-TCR is lower in mutant mice, suggesting that LAT controls the expression of the pre-TCR complexes of DN thymocytes. Signal transduction through the pre-TCR and through the clonotype independent complexes (CICs, consisting of calcineurin and either CD3-γδ or CD3-δε pairs) expressed at the membrane of wild type or RAG-deficient DN cells, can be triggered by intra-peritoneal injection of anti-CD3 antibodies. However, anti-CD3 stimulation of LAT−/− and LATY7/8/9F DN thymocytes induced neither proliferation nor differentiation to the DP stage(33). In contrast, the same treatment in LATY7/8/9F mice induced a modest proliferation and expression of CD4 and CD8 of DN thymocytes, suggesting that, under supra-physiological conditions LATY7/8/9F molecules are still able to transduce signals. This result is in agreement with previous data generated in Jurkat cells, and can be explained by a lack of recruitment of the SLP-76 adaptor by LATY7/8/9F mutant molecules. Indeed, intra-peritoneal injection of anti-CD3 antibodies in SLP76−/− mice also induced the development of a few DP cells as it happened in LATY7/8/9F mice(34).

Furthermore, in order to address the importance of tyrosine 6 of LAT in vivo and to analyze the consequence of restricting LAT to only a subset of its docking functions, we and others generated knock-in mice with a mutation that replaced tyrosine number 6 (also called Tyr 136) with phenylalanine (LATY6F(35,36). The thymi of LATY6F mice had about one-tenth the cells of wild type thymi and reduced numbers of DP thymocytes. After reaching a peak in mutant newborn mice, DP cell numbers decreased and were almost undetectable in mice older than 7 weeks. Coincident with this progressive DP cell erosion, discrete populations of CD4 and CD8 SP cells started to dominate the thymus and showed a CD4/CD8 ratio skewed toward CD4 cells. The phenotype of these CD4 T cells (CD44high, CD62Llow, CD69−, and CD24+) was distinct from that expected for genuine CD4 SP thymocytes. These CD4 T cells corresponded to abnormal peripheral CD4 cells that expanded in the periphery of LATY6F mice, and infiltrated the thymus. The cytokines interleukin-5 (IL-5) and IL-13 they produced in situ are responsible for thymic eosinophilia and fibrosis, resulting in the subsequent erosion of the DP cell compartment. Moreover, the DP thymocytes found in young mutant mice lacked CD5 molecules at their surface. CD5 is a negative regulator of TCR signaling, and its expression increases during T cell development in a manner proportional to the intensity of pre-TCR and TCR signaling(37-39). Consistent with that hypothesis, the small complement of DP cells characteristic of young LATY6F mice remained stable over time when development of the infiltrating, CD4 T cells was prevented by breeding the LATY6F mutation on a genetic background deprived of MHC class II molecules(30). Thus, from these data and the presence of a normal complement of DN cells in LATY6F thymuses, it can be concluded that mutation of tyrosine 6 of LAT results in a severe but partial impairment of αβ T cell development.

A recently identified transmembrane adaptor protein called NTAL (for non-T cell activation linker) or LAB (for linker of activation of B cells) is structurally and evolutionarily related to LAT(40,41). NTAL is expressed by B lymphocytes, NK cells, monocytes, and mast cells but not by resting T lymphocytes, and is the product of a previously identified Wbscr5 gene of so far unknown function. NTAL, which possesses a short extracellular domain, a transmembrane segment, two palmitoylated cysteines and a long cytoplasmic tail, becomes rapidly tyrosine-phosphorylated upon ligation of immune receptors of B cells and mast cells. Regardless of the remarkable conservation of the exon-intron structure of the Ntal and Lat genes (suggesting that both come from a duplication of a common precursor gene), there are important differences in the molecular partners they can bind. Strikingly,
Although both adaptors show in their sequence several consensus motifs for the binding of the SH2 domain of Grb2, only LAT shows a motif for the binding of PLC-γ1. Therefore, NTAL is not able to bind PLC-γ1, resembling LAT-Y6F mutant molecules. Indeed, a NTAL transgene expressed in a LAT-deficient background was able to restore T cell development, but these NTAL transgenic mice showed a phenotype strikingly similar to that of LAT-Y6F mice. Moreover, by means of using recombinant retroviruses expressing different mutant forms of LAT in a LAT-/- background, Zhang and co-workers have shown that a form of LAT with only the last C-terminal tyrosine residues behaves similarly to that of the LAT-Y6F mutant mice. Therefore, this demonstrates that the five NH2-terminal tyrosines of LAT (LAT tyrosines 1 to 5) are dispensable for the pathology developed by the LAT-Y6F mutation.

**ROLE OF LAT IN γδ T CELL DEVELOPMENT**

T lymphocytes can be divided into two subsets based on the structure of the TCRs expressed at their surfaces. As mentioned above, during thymic development αβ T cells have to cross two sequential checkpoints in order to assure the survival of only those cells that have properly rearranged TCRβ and -α genes and expressed a αβ TCR with appropriate affinity. In the same way, during their development, thymocytes committed to the γδ lineage encounter a unique checkpoint at the DN (CD44 CD25-) stage, allowing only cells that have properly rearranged γδ TCR to mature into CD44 CD25 CD4- CD8- γδ T cells and to leave the thymus (Fig. 3). The signal transduction machinery operated by the pre-TCR, αβ and γδ TCR complexes share many components. As described above, in vitro experiments and data obtained from the analysis of LAT deficient or knock out mice have revealed that this adaptor is essential for a proper coordination of intracellular signals coming from the pre-TCR and the αβ and γδ TCRs. Indeed, development of T cells bearing TCR made of γδ chains is totally ablated in LAT-/- mice.

In order to address the role of the four distal tyrosines (6, 7, 8 and 9) of LAT in γδ T cell development, we studied this process in a series of mutant animals in which these tyrosine residues have been substituted by phenylalanines (see above). TCR γδ cells were readily detected within the thymus of young LAT-Y7/8/9F mice, but, in contrast to previous reports, CD3- γδ TCR- cells were also present in LAT-/- and LAT-Y6/7/8/9F thymi. Therefore, some γδ thymocytes can emerge in the absence of LAT, or in mice expressing LAT-Y7/8/9F or LAT-Y6/7/8/9F molecules, but only the γδ T cells found in LAT-Y7/8/9F thymi reached the periphery where they expanded and gave rise, as described below, to a lymphoproliferative disorder.

The phenotype of γδ thymocytes found in LAT-Y7/8/9F is different from that expected for genuine γδ thymocytes, since they express lower levels of CD5 molecules and close to 90% of γδ LAT-Y7/8/9F thymocytes expressed the CD25 molecule (whereas only a small fraction of wild type γδ thymocytes are CD25+). Thus, since it does not prevent the surface expression of γδ TCR, the LAT-Y7/8/9F mutation contrasts with null mutations involving the CD3 subunits, providing a unique opportunity to visualize a transitory CD25+ TCR γδ population. Although the LAT-Y7/8/9F mutation allows the development of some of the γδ T cell subsets normally present in the periphery, it prevents the development of dendritic epithelial T cells (a population of γδ cells that resides in the epidermis), and of the CD8αα TCR γδ intraepithelial lymphocytes in the gut. Therefore, it seems that different signaling thresholds are needed for αβ and γδ development, and the development of the various γδ T cell subsets differs in their dependence on LAT signaling.

**REGULATORY FUNCTIONS OF LAT**

αβ T cells in LAT-Y6F mice

Unexpectedly, although there are very few SP thymocytes in newborn LAT-Y6F mice, secondary lymphoid organs of these animals present a population of CD4 T cells that accumulate over time. This population shows a strong bias for CD4 T cells, expresses low levels of TCR and has a CD25+, CD44hi, CD62Llo, CD69+ phenotype, closely resembling activated and memory T cells. The analysis of this population of cells has shown extended survival and increased proliferation, features that account for their progressive accumulation. Paradoxically, these CD4 T cells are refractory to in vitro stimulation through the TCR, an attribute that can be explained by the low levels of TCR expressed at their membranes. Interestingly, when freshly isolated from LAT-Y6F mice, CD4 T cells express high levels of IL-4 and IL-10 transcripts without ex vivo stimulation. Upon activation by phorbol 12-myristate 13-acetate (PMA) plus ionomycin, IL-5, IL-13, and interferon-γ (IFN-γ) transcripts are also detected. In contrast, wild-type CD4 T cells only show the IL-2 and IFN-γ transcripts expected after activation of primary T cells. Thus, over the first weeks of their life, LAT-Y6F mice spontaneously develop a T helper type 2 (TH2) lymphoproliferative disorder. In the case of wild-type CD4 T cells, a TH2 polarization of such magnitude is only achieved after prolonged antigenic stimulation in the presence of IL-4.
Proximal activation events, such as tyrosine phosphorylation of TCRζ and ZAP-70, are unaffected in LATY6F T cells(36). However, tyrosine phosphorylation of LAT and of PLC-γ1 is markedly reduced in LATY6F T cells after antibody stimulation. TCR cross-linking also fails to mobilize calcium or activate the calcineurin-dependent transcription factors NF-ATc1 and NF-ATc2. Unexpectedly, given the low level of LAT tyrosine phosphorylation upon TCR engagement, tyrosine phosphorylation of SLP-76, which binds indirectly to LAT tyrosines other than 6, is unimpaired. Surprisingly, and in contrast to the results obtained in T cell lines(9,13,19), Erk activation in response to CD3 plus CD4 cross-linking is normal or only slightly reduced in CD4+ T cells and in DP thymocytes from LATY6F mice. Therefore, despite their low levels of tyrosine phosphorylation following TCR stimulation, LATY6F molecules seem able to recruit enough Grb2-Sos complexes to activate the Ras-Erk pathway. Alternatively, the generation of active PLC-γ1 could be achieved in LATY6F mice via a «trickle through» mechanism(45,46). Finally, it is also possible that under these conditions, Erk activation is mediated independently of LAT.

Secondary lymphoid organs of adult LATY6F mice contain 7 to 10 times as many B cells as their wild-type counterparts. The majority of these cells show a hyperactivated phenotype or a phenotype typical of antibody-producing cells, while most of B cells in wild type mice have a resting phenotype. Serum IgG1 and IgE concentrations are highly elevated compared with wild-type mice, although levels of other immunoglobulin isotypes do not differ from those of wild-type mice. The splenomegaly and hypergammaglobulinemia of LATY7/8/9F mice is unimpaired. Surprisingly, and in contrast to the results documented for LATY136F mice(53), LATY7/8/9F mice develop lymphoproliferative disorders characterized by the development of hypergammaglobulinemia E and G1 mutations acts primarily at the level of CD4 T cells, and the development of the hypergammaglobulinemia E and G1 does not require the expression of LATY6F molecules within B cells.

**γδ T cells in LATY6F mice**

Surprisingly, over 90% of LATY7/8/9F mice older than twenty weeks exhibit enlarged lymph nodes and spleens containing an expanded population of γδ T cells and elevated numbers of B cells(30). Given the small size of the γδ T cell population found in young LATY7/8/9F thymi (see above), the presence of large numbers of γδ T cells in the spleen and lymph nodes of older LATY7/8/9F mice suggests that these cells have arisen by proliferative expansion in the periphery. Such a lymphoproliferative disorder has never been documented for γδ T cells developing in the mere absence of αβ T cells, as it happens in TCRβ−/− mice(52) or β-deficient mice(53). Therefore, this disorder cannot be accounted for by the lack of an extrinsic negative loop originating from regulatory αβ T cells.

The γδ T cells expanding in LATY7/8/9F mice also show a CD25−, CD44+, CD62L−, CD69+ phenotype, they are primarily CD4− CD8− or CD4+, and express levels of TCR lower than those found on wild-type γδ T cells. Biochemical and histological analysis have confirmed the oligoclonal and non-malignant nature of this population. In addition, upon activation by PMA and ionomycin, this cell population expresses IL-4, IL-5, IL-10, and IL-13 transcripts in amounts comparable to those found in αβ CD4 T cells freshly isolated from LATY6F mice. Therefore, the lymphoproliferative disorders characterizing LATY7/8/9F and LATY6F mice are each associated with TH2 polarization. Similarly to CD4 T cells that expand in LATY6F mice, the γδ T cells of LATY7/8/9F mice also fail to proliferate in response to CD3 cross-linking.

As expected from the pattern of interleukins produced by the expanding γδ T cells, the spleen and the lymph nodes of LATY7/8/9F mice older than 20 weeks contain 5 times as many B cells as their wild-type counterparts and show a hyperactivated phenotype or a phenotype typical of antibody-producing cells. Again, serum IgG1 and IgE concentrations are elevated about 500 and 1000 times, respectively, compared with those of wild-type mice. The splenomegaly and lymphadenopathy that develop in LATY7/8/9F mice is therefore mostly accounted for by expansion of cells belonging to the γδ T cell and B cell lineages.

**A negative regulatory role for LAT**

In T cell lines, most of the signaling activity of LAT is channeled through the four carboxy-terminal tyrosine residues(6,13,15). In support of these data, a LATY6F/7/8/9F mutant
behaves in vivo as a LAT null, and blocked both γδ and αβ T cell development\(^{31,32}\). Furthermore, in agreement with a positive role for LAT in signal transduction, mutation of tyrosine 6 (LAT\(^{Y6F}\)) or the three distal tyrosines (LAT\(^{Y7/8/9F}\)) hampers and prevents αβ T cell development, respectively. However, once phosphorylated, LAT molecules may participate in activating negative regulatory mechanisms at later time points. Indeed, it has been shown that, upon its phosphorylation, LAT recruits the cytosolic adaptor Gab2, resulting in the mobilization to the LAT complex of inhibitory molecules as the SHP-2 tyrosine phosphatase\(^{54}\). In this context, the mutant LAT\(^{Y6F}\) and LAT\(^{Y7/8/9F}\) molecules are still able to transduce low intensity signals allowing the development of a few αβ or γδ T cells. Nevertheless, the signaling capability of these mutant forms of LAT could be not strong enough for triggering the negative loop expected to arrest TCR mediated signaling. Thus, given that the TCR encounter with self-peptide/MHC complexes triggers intracellular signals that sustain naive T cells in an optimal state of sensitivity and allow their survival in the periphery\(^{35}\), the LAT\(^{Y6F}\) and LAT\(^{Y7/8/9F}\) mutations would increase the duration and magnitude of such signals. This model is congruent with the recessive character of LAT\(^{Y6F}\) and LAT\(^{Y7/8/9F}\) mutations: in heterozygous mice signals transduced by the mutant forms of LAT are likely blunted by dominant, negative signals originating from wild type LAT molecules colocalizing in the GEMs. It has been recently shown that LAT can be phosphorylated on serine and threonine residues, and it has been proposed that phosphorylation of threonine 155 of human LAT by Erk kinase could negatively regulate PLC-γ1 binding\(^{50}\). Indeed, reconstitution of LAT deficient J.CaM2 cells with a T155A mutant increases TCR-induced Ca\(^{2+}\) influx generation. Therefore, as previously documented, the Ras-Erk pathway may both promote and attenuate TCR signaling\(^{57}\). However, since threonine 155 residue is not conserved in all the other species analyzed, it remains to be determined whether LAT Ser/Thr-phosphorylation by Erk constitutes a general negative regulatory mechanism controlling TCR signals.

CONCLUDING REMARKS

LAT is not only the first transmembrane adaptor protein described, but it also seems to be the main device switching on TCR and pre-TCR intracellular signals. In vitro experiments have demonstrated that LAT has a crucial role in the transduction of early signaling events, and have revealed the importance of several of its tyrosine residues in the distribution of signals to different downstream signaling pathways. Nevertheless, in spite of the significance of the information obtained from Jurkat cells, in vivo studies have discovered unexpected regulatory functions for this adaptor. From the analysis of either LAT\(^{Y6F}\) or LAT\(^{Y7/8/9F}\) mice it seems that LAT contributes to a non-characterized negative loop of intracellular signaling. In such a way, the diminished signaling capabilities of LAT\(^{Y6F}\) or LAT\(^{Y7/8/9F}\) would be enough to transduce signals for the maturation of a few αβ or γδ cells, but not for triggering the negative signals. Thus, rather than disrupting discrete signaling pathways from the TCR, these mutations may impair the formation of the quaternary complex consisting of LAT, Gads, SLP-76 and PLC-γ1. Therefore, the phenotype shown by LAT\(^{Y6F}\) or LAT\(^{Y7/8/9F}\) mice may be the consequence of quantitative reduction of signals rather than specific changes in discrete downstream signaling pathways. The underlying mechanisms by which LAT\(^{Y6F}\) or LAT\(^{Y7/8/9F}\) mutations impact on T cell development and differentiation remain to be defined. In this regard, proteomic and genomic approaches intending to discover the interaction of LAT with other membrane and cytosolic adaptors and to define the functional consequences of these mutations could shed some light in this puzzling issue.

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