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A Tale of Two TRAPs: LAT and LAB in the Regulation of Lymphocyte Development, Activation, and Autoimmunity

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Abstract

Transmembrane adaptor proteins (TRAPs) link antigen receptor engagement to downstream cellular processes. Although these proteins typically lack intrinsic enzymatic activity, they are phosphorylated on multiple tyrosine residues following lymphocyte activation, allowing them to function as scaffolds for the assembly of multi-molecular signaling complexes. Among the many TRAPs that have been discovered in recent years, the LAT (linker for activation of T cells) family of adaptor proteins plays an important role in the positive and negative regulation of lymphocyte maturation, activation, and differentiation. Of the two members in this family, LAT is an indispensable component controlling T cell and mast cell activation and function; LAB (linker for activation of B cells), also called NTAL, is necessary to fine-tune lymphocyte activation and may be a key regulator of innate immune responses. Here, we review recent advances on the function of LAT and LAB in the regulation of development and activation of immune cells.

Keywords

TRAP (transmembrane adaptor protein); tyrosine phosphorylation; autoimmunity; lymphoproliferative disease; T cell homeostasis; PLC- γ

Introduction

Antigen receptors, the T cell receptor (TCR) or the B cell receptor (BCR), are vital for the integration of different extracellular signals that ultimately result in the activation of transcription factors and drive lymphocyte development, differentiation, and activation. Upon receptor engagement, Src family kinases, such as Lck, Lyn, and Fyn, are activated and phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic tails of antigen receptor-associated subunits. Phosphorylated ITAM motifs serve as docking sites for the Syk family kinases, Syk and Zap-70, in B and T cells, respectively. Syk and Zap-70 are then phosphorylated and activated by the Src kinases, initiating a cascade of downstream signaling pathways by phosphorylating other proteins in close proximity to the receptor complex. A vital element necessary for bridging the initial receptor engagement to the downstream signaling events is the transmembrane adaptor protein (TRAP). TRAPs are critical for the assimilation of extrinsic signals to a cellular output by organizing multi-molecular protein complexes at the plasma membrane (1, 2).

Several TRAPs have been discovered in recent years and their roles in antigen receptor-mediated signaling have been highlighted in studies using cell lines and genetically-modified mice. Although these proteins typically have no intrinsic enzymatic activity, TRAPs are tyrosine phosphorylated and interact with other signaling proteins that contain SH2-domains, thus serving as scaffolds to recruit downstream effector proteins. Among all

TRAPs, LAT (linker for activation of T cells) has been shown to be the most important one in hematopoietic cells. Studies using LAT-deficient Jurkat cells and LAT knockout mice indicate that LAT is essential during thymocyte development and T cell activation (3, 4). LAT is also critical in FcεRI-mediated signaling and mast cell function (5). While LAT plays an indispensable role in T cells and mast cells, LAB (linker for activation of B cells)/NTAL (non-T cell activation linker), the other member of the LAT family, has been shown to be important to fine-tune the activation and function of T cells and mast cells (6–9). LAT and LAB are able to exert both positive and negative effects on the signaling pathways that regulate the activation and function of a variety of hematopoietic cells.

In addition to the LAT family of adaptor proteins, other TRAPs play key roles in immune receptor signaling. For example, SIT (SH2 domain-containing phosphatase 2-interacting TRAP) and TRIM (TCR-interacting molecule) negatively regulate TCR-mediated signals. The loss of both adaptors leads to enhanced positive selection during thymocyte development (10). PAG (phosphoprotein associated with glycosphingolipid-enriched domains), a ubiquitously expressed TRAP, is able to inhibit Src kinase activity and Ras activation through its recruitment of Csk and RasGAP to the membrane (11–13). LIME (Lck-interacting molecule) is an adaptor protein found in hematopoietic cells known to associate with Lck, Gads (Grb2-related adaptor protein), and Grb2 (14). This review, however, will focus on the more recent advances from studies concerning the roles of LAT and LAB in immune receptor signaling.

LAT

LAT is a transmembrane protein containing a short extracellular domain, transmembrane domain, and cytoplasmic tail with nine conserved tyrosine motifs. It is expressed in T cells, NK cells, mast cells, megakaryocytes, and pre-B cells (15–18). The functional importance of LAT in TCR-mediated signaling has been clearly demonstrated in LAT-deficient Jurkat T cells. TCR-mediated calcium mobilization, Erk activation, CD69 upregulation, and AP-1 and NFAT-mediated gene transcription are severely impaired in these cells (3, 19). As an adaptor protein, the function of LAT in TCR signaling centers upon its tyrosine phosphorylation and subsequent recruitment of other signaling proteins. Upon TCR engagement, phosphorylation of LAT allows it to interact with several SH2 domain-containing proteins, such as Grb2, Gads, and PLC-γ1 (15, 20). Studies performed by reconstituting LAT-deficient Jurkat cells with LAT mutants unable to bind PLC-γ1 and Grb2 show the necessity of the association of LAT with both of these proteins for TCR-mediated Ras activation, calcium flux, and NFAT activation (21, 22). LAT also contains two sites for binding Gads. Through its constitutive interaction with SLP-76, the recruitment of Gads to LAT brings SLP-76, a cytosolic adaptor protein, into close proximity of LAT (20). The binding of LAT and Gads is indispensable for the full activation of T cells, although reconstitution of LAT-deficient Jurkat cells with a LAT mutant unable to bind Gads shows a partial rescue of calcium flux and NFAT activation. Our data indicate that Grb2, Gads, and PLC-γ1 bind cooperatively to LAT (23). We believe that Grb2 may stabilize the Gads-LAT and PLC-γ1-LAT interactions, which can be confirmed by solving the three dimensional structure of the LAT-Grb2-Gads complex. Although LAT is likely an unstructured molecule (unpublished data), it is certainly feasible to generate a trimolecular complex of Grb2, Gads, and phosphorylated LAT peptide for crystallization and further structure determination.

LAT palmitoylation and raft localization

The juxtamembrane region of LAT contains two cysteine residues that are required for LAT palmitoylation, raft localization, phosphorylation, and function in TCR-mediated signaling (24). To further study the importance of LAT localization to lipid rafts, we reconstituted

LAT-deficient Jurkat cells with a LAX-LAT construct that consists of the extracellular and transmembrane portions of LAX and the intracellular domain of LAT. LAT is a transmembrane adaptor that is not localized to lipid rafts. Even though this fusion protein is partitioned into lipid rafts, it can rescue the signaling defects in these cells. Moreover, this LAX-LAT fusion protein can replace LAT in thymocyte development (25). While our data clearly demonstrate that LAT localization to lipid rafts is not an absolute requirement for its function in TCR-mediated signaling, a recent follow-up study argues that this LAX-LAT protein is localized in a newly defined “heavy fraction” of the plasma membrane, even though it is not localized in lipid rafts. Interestingly, it was shown that a CD25-LAT fusion protein, which is not localized in either lipid rafts or the heavy fraction, is also able to support TCR signaling, albeit at a reduced level (26). These data further support our previous conclusion that LAT localization to lipid rafts is not essential for its function in TCR-mediated signaling. It is certainly possible that LAT may function more efficiently within lipid rafts.

Although the physiological relevance of LAT localization to lipid rafts remains to be determined, LAT palmitoylation is undeniably essential for its function and may be regulated during T cell activation, differentiation, or anergy induction. One study shows that LAT palmitoylation is impaired in anergic T cells, leading to defects in the signaling events downstream of LAT, such as PLC- γ 1 phosphorylation and calcium mobilization, while signaling events upstream of LAT, such as ZAP-70 phosphorylation, remain intact in these cells (27). These data indicate that T cell function can be regulated through LAT palmitoylation.

LAT in thymocyte development

LAT-deficient mice contain normal B cell populations but completely lack peripheral T cells. They have an early block at the DN3 stage of thymocyte development, resulting in the complete absence of DP and SP thymocytes, as well as $\gamma\delta$ T cells. These studies demonstrate the essential role of LAT in pre-TCR signaling during thymocyte development (4). Due to the severe block at the DN3 stage in LAT-deficient mice, the role of LAT in later stages of thymic development could not be elucidated from the analysis of these mice. Therefore, our lab recently generated LAT knock-in mice in which the *lat* gene can be deleted by the Cre recombinase. Deletion of LAT by Cre under the control of the CD4 proximal promoter allows for the generation of DP thymocytes; however, the development of SP thymocytes is severely blocked in these mice. Therefore, LAT plays an irreplaceable role in both the early and late stages of thymic development (28).

LAT in T cell homeostasis

One of the exciting findings involving LAT in recent years is that mutation of the PLC- γ 1 binding site on LAT (Y136) leads to a severe lymphoproliferative disease in mice. Experiments using LAT-deficient Jurkat T cells expressing the LATY136F mutant indicate that Y136 is required for LAT binding with PLC- γ 1. Mutation of this tyrosine impairs TCR-mediated calcium flux and blocks Erk and NFAT activation. Therefore, the LAT-PLC- γ 1 interaction is critical for the emanation of signals originating from the TCR (21, 22).

To investigate the importance of the LAT-PLC- γ 1 interaction *in vivo*, two groups independently generated knock-in mice that express the LATY136F mutant (29, 30). In agreement with the *in vitro* data showing the partial function of this mutant in TCR-mediated signaling, LATY136F mice have a partial block at the DN3 stage; however, a small percentage of cells are able to mature into DP and SP cells. Surprisingly, T cells in these mice are hyperactivated and undergo a huge expansion, causing a fatal lymphoproliferative autoimmune disease. The autoimmune disease observed in LATY136F

mice is T_H2-skewed, characterized by tissue eosinophilia and massive production of IgE and IgG₁ by B cells. These mice exhibit splenomegaly, lymphadenopathy, and lymphocyte infiltration in the lung, liver, and kidney. T cells in these mice are predominantly CD4⁺ and exhibit a memory/activated phenotype with high surface expression of CD44 and downregulated expression of CD62L. Interestingly, TCR surface expression in mutant T cells is reduced. TCR-mediated LAT and PLC- γ 1 phosphorylation and calcium flux are impaired. Furthermore, these T cells are resistant to TCR-mediated cell death. In addition, B cells from these mice are also hyperactivated, resulting in elevated autoantibody serum titers and systemic autoimmunity marked by the incredible production of IgE and IgG₁. While LAT has long been established as a positive regulator of T cell activation, these findings raise the possibility that LAT has a negative role in TCR-mediated signaling.

A striking characteristic of the LATY136F lymphoproliferative disease is the tremendous amount of IL-4 produced by T_H2 cells in these mice. Interestingly, a recent study indicates that the lymphoproliferative disease is not dependent upon the assumption of a T_H2 lineage fate. In the absence of STAT6, the transcription factor responsible for IL-4 production and T_H2 differentiation, STAT6^{-/-} LATY136F mice also develop a lymphoproliferative disease of the same timing and severity as LATY136F mice. However, this disease is instead marked by IgG_{2a} and IgG_{2b} hyperagammaglobulinemia and involvement of CD8⁺ and T_H1 cells (31). Therefore, regardless of cytokine profiles and T helper cell differentiation, the lymphoproliferative disease that develops in these mice is dependent upon intrinsic T cell defects that result from the abrogation of the LAT-PLC- γ 1 interaction.

Similar to LATY136F mice, LAT3YF mice, which harbor mutations at tyrosines 175, 195, and 235, contain a form of LAT protein with only partial docking function. While PLC- γ 1 may potentially still bind to the Y136 residue upon phosphorylation, mutation of these other three residues abolishes LAT association with Gads and Grb2. LAT3YF mice show a complete block in $\alpha\beta$ T cell development but accumulate $\gamma\delta$ T cells in peripheral lymphoid organs, suggesting a differential requirement of LAT in $\alpha\beta$ and $\gamma\delta$ T cell development. Unexpectedly, by five months of age, these mice develop a severe lymphoproliferative disease characterized by T_H2-like $\gamma\delta$ T cells and IgG₁/IgE hyperagammaglobulinemia (32).

The severe autoimmunity observed in LATY136F mice could be a consequence of defective negative selection. Indeed, further analyses of LATY136F mice using the HY-TCR transgenic system, which is routinely used to examine positive and negative selection, show that both selection processes are severely impaired. Thus, it is possible that autoreactive T cells, which would normally be deleted, are now able to escape central tolerance, perhaps contributing to their uncontrolled expansion in the periphery (33). Interestingly, these LATY136F T cells are still able to expand in MHC class II-deficient hosts, implying that the hyperproliferation of these cells is independent of the TCR-MHC interaction (34).

In addition to the impaired thymic selection in LATY136F mice, our results show that these mice fail to develop natural CD4⁺CD25⁺ T regulatory cells (T_{regs}). In thymocytes and mature T cells from these mice, Foxp3 expression is significantly reduced at the RNA level and Foxp3 protein expression is not detectable by intracellular antibody staining. Upon adoptive transfer of normal T_{reg} cells into neonatal LATY136F mice, the mice fail to develop the autoimmune disease. Indeed, the presence of T_{reg} cells stymies the expansion of LATY136F T cells. These data highlight the importance of CD4⁺CD25⁺ T_{regs} in the suppression of the LATY136F autoimmune syndrome (35).

In contrast to our results, Wang *et al.* employed a system using *Foxp3EGFP* reporter mice to study the contribution of T_{regs} to the LATY136F phenotype (34). Interestingly, their study shows that Foxp3⁺ T cells are actually present in LATY136F mice but are nonfunctional.

While their data apparently contradict our data, we speculate that this is due to the sensitivity of methods used to detect Foxp3 expression. The *Foxp3EGFP* reporter system is probably more sensitive due to the highly stable nature of EGFP. Collectively, it is most likely that Foxp3 expression in T_{reg} cells is greatly impaired, but not totally abolished, by the LATY136F mutation.

Our laboratory has recently been working to further characterize T_{reg} cells in the LATY136F mice. We have developed an inducible deletion system in which a wildtype LAT allele allows for proper T cell development but can be deleted in the periphery upon injection of tamoxifen. After deletion of the floxed wildtype allele, T cells are left with only a copy of the mutated LATY136F LAT allele. Our data show that injection of tamoxifen induces the development of autoimmune disease similar to that seen in the LATY136F mice. Thus, the Y136F mutation in mature T cells alone is sufficient for the development of the severe lymphoproliferative disease in LATY136F mice, suggesting that defective negative selection in LATY136F mice may not be the only cause for autoimmunity. Although Foxp3⁺ regulatory T cells can be found in these mice, they are impaired in their ability to suppress the proliferation of conventional T cells *in vitro*. They also have significantly decreased CTLA-4 surface expression and IL-10 and TGF- β production (36). While it is clear that regulatory T cells are nonfunctional in the absence of the LAT-PLC- γ 1 interaction, the precise mechanisms governing these responses have yet to be uncovered. Interestingly, while mice expressing the LATY136F mutant develop the autoimmune syndrome as discussed above, mice lacking LAT in mature T cells also develop a similar autoimmune syndrome, although the disease observed is much less severe than in LATY136F mice (37). LAT-deficient CD4⁺ T cells can undergo expansion and secrete large amounts of cytokines despite a severe defect in TCR-mediated signaling. Together, the data from analyses of these LAT mutant mice clearly indicate the critical role of this adaptor protein in T cell homeostasis. The mechanism by which LAT controls T cell homeostasis is yet to be recovered.

LAB

Because LAT plays an undeniably essential role in T cell development and T cell activation, we speculated that an equivalent protein might exist in other immune cells, particularly B lymphocytes. The search for LAT-like adaptor proteins led to the discovery and cloning of LAB (linker for activation of B cells), also known as NTAL (non-T cell activation linker). Although LAB does not share any major sequence homology with LAT, it has similar structural features. LAB contains a short extracellular domain, a transmembrane domain, and a cytoplasmic tail with multiple tyrosine residues. LAB also has a CxxC palmitoylation motif and is localized to lipid rafts. Although LAB protein is not detected in the thymus or in naïve T cells, it is highly expressed in B cells, mast cells, monocytes, and NK cells (6, 7).

Upon engagement of the BCR, Fc γ RI, or Fc ϵ RI, LAB is phosphorylated most likely by Syk kinase. As predicted from its five Grb2 binding motifs, LAB associates with Grb2, as well as Sos, Gab1, and Cbl. However, unlike LAT, LAB does not have a PLC- γ binding motif and is not able to interact with PLC- γ 1 or PLC- γ 2. Furthermore, reconstitution of a LAT-deficient Jurkat cell line with LAB leads to a partial rescue of Erk activation and calcium flux (6, 7). Considering the importance of tyrosine phosphorylation in LAT function, we generated a series of LAB mutants in which the nine conserved tyrosine residues were mutated into phenylalanines to map the phosphorylation sites and to determine which ones are required for LAB function. Our results show that LAB phosphorylation mainly occurs on the three membrane-distal tyrosines, Tyr136, Tyr193, and Tyr233. These residues are most important for Grb2 binding and for LAB function in the rescue of calcium flux and

thymocyte development in LAT-deficient mice (38). Again, similar to LAT, the intrinsic function of LAB relies heavily upon the phosphorylation of its membrane-distal tyrosines.

To further study the functional differences between LAT and LAB, we generated transgenic mice expressing LAB under the human CD2 promoter. LAB can partially rescue thymocyte development in the LAT-deficient mice. Although there is an accumulation of thymocytes at the DN3 stage, a moderate percentage of thymocytes are able to progress into the DP and SP stages. In the periphery, there are significant numbers of both CD4⁺ and CD8⁺ cells, although the CD4 to CD8 ratio is vastly skewed in favor of CD4⁺ T cells. Furthermore, by 10 weeks of age, LAB-transgenic mice develop an autoimmune syndrome similar to that seen in LAT^{Y136F} mice. CD4⁺ T cells also undergo uncontrolled expansion and they have organomegaly with excessive lymphocytic infiltration into different organs. (39). From these results, we concluded that LAB is a functional equivalent of LAT without the PLC- γ 1 binding site.

LAB in B cells

Even though LAB is highly expressed in B cells and is phosphorylated upon BCR ligation, B cell development in LAB^{-/-} mice is surprisingly normal (40, 41). These mice have normal numbers of T1, T2, and follicular B cells with only a reduced number of marginal zone B cells. LAB-deficient B cells actually demonstrate a slightly enhanced calcium response while MAPK activation and humoral responses are unaffected. While these studies show that LAB in B cells does not play an analogous role to LAT in T cells, studies using the DT40 chicken B cell line suggest that LAB is important in BCR-mediated calcium mobilization (42). It is hypothesized that, upon its association with LAB, Grb2 is recruited to lipid rafts and can no longer associate with a yet to be characterized Ca²⁺ inhibitor. While the data from this paper appear to be interesting, whether LAB indeed has a significant role in BCR-mediated calcium flux in B cells or even in DT40 cells remains unclear as LAB expression in DT40 cells is very low (unpublished observation). Recent data suggest that LAB is important in the internalization of the BCR (43, 44). While BCR signal transduction itself leads to an increase in the surface expression of MHC class II, CD80, and CD86, B cells rely upon endocytosis to present antigens to T cells. BCR endocytosis has been shown to require Vav1 and Vav3 as this process is blocked in B cells from Vav1/3^{-/-} mice. Interestingly, LAB^{-/-} B cells have a defect in their ability to internalize the BCR. BCR-mediated Vav phosphorylation and Rac activation are reduced in LAB^{-/-} B cells. Furthermore, LAB-mediated BCR internalization depends upon its association with Vav and dynamin (43, 44). Together, these data indicate that LAB does function in BCR-mediated signaling pathways, although its role is distinct from the role of LAT in T cells.

LAB in T cells

Despite normal lymphocyte development in LAB^{-/-} mice, aged LAB-deficient mice develop an autoimmune disease characterized by splenomegaly and anti-nuclear antibodies. While B cells in these mice are relatively normal, T cells are hyperactivated and produce high levels of cytokines, such as IL-2, IL-10, and IFN- γ . These results were highly unexpected since LAB is not expressed in naïve T cells. However, further analysis revealed that LAB is upregulated in T cells after TCR stimulation. Comparisons of LAB^{-/-}, LAB^{Tg}, and wildtype cells demonstrate that the loss of LAB leads to enhanced LAT, PLC- γ 1, Akt, and Erk phosphorylation, as well as increased calcium flux. Conversely, the transgenic expression of LAB resulted in the diminution of most TCR-mediated signaling events. Analysis of LAT localization to lipid rafts in these cells shows that LAB^{-/-} cells contain more LAT protein in lipid raft fractions, while LAB^{Tg} cells have significantly decreased levels of LAT in these microdomains. These results imply that LAB may exert its negative

regulatory function on T cell signaling by competing with LAT for palmitoylation and/or lipid raft localization. Interestingly, when crossed onto a LAT^{Y136F} background, LAB deficiency further enhances the lymphoproliferation and organomegaly caused by dysfunctional T cells (9). Together, these data demonstrate a role for LAB in regulating T cell activation and limiting autoimmune responses. In addition, LAB is also found to be highly expressed in TCR $\alpha\beta$ ⁺CD8 $\alpha\alpha$ and TCR $\gamma\delta$ ⁺CD8 $\alpha\alpha$ intraepithelial lymphocytes (IEL) from mouse small intestines. These IELs are also self-reactive T cells and relatively anergic. Different from conventional T cells, they express NK receptors and may use a different signaling apparatus. It is speculated that these cells are potent regulators in the epithelium (45). Interestingly, these IELs have very low levels of LAT mRNA; however, they express an amount of NTAL/LAB mRNA that is nearly identical to the splenic B cells. How LAB functions in these cells remains to be determined.

LAB in innate immunity

The initiation of an innate immune response requires the engagement of receptors on the cell surface with invading pathogens. TREM-1 (triggering receptor expressed on myeloid cells), a glycoprotein on the surface of myeloid cells, is an activating receptor known to interact with DAP12 (DNAX activation protein of 12kDa). DAP12 is an important transmembrane adaptor protein that non-covalently associates with several receptors and contains ITAM motifs. These ITAM motifs are phosphorylated upon the engagement of TREM-1 by its ligands, which are as of yet unidentified, leading to the activation of PLC- γ and Erk. Recent data show that engagement of TREM-1 results in LAB phosphorylation. Experiments knocking down LAB protein expression in myelomonocytic cell lines indicate that LAB negatively modulates TREM-1-mediated Erk1/2 activation, calcium flux, and production of TNF- α and IL-8 (46). Similar to TREM-1, TREM-2, which induces the upregulation of co-stimulatory molecules and chemokine receptors on dendritic cells, utilizes DAP12 to propagate its signal (47). One recent study shows that LAB is required for TREM-2-mediated activation of Erk1/2. LAB^{-/-} macrophages have increased TREM-2-induced phosphorylation and, upon LPS stimulation, these deficient macrophages produce increased levels of IL-10 and decreased amounts of IL-12 (48). It seems certain that, as more details concerning innate signaling pathways are illuminated, LAB will emerge as an important regulator of innate immune responses.

LAT and LAB in mast cells

The critical role of LAT and LAB as adaptor proteins is not limited to lymphocytes and indeed extends to Fc ϵ RI-mediated signaling in mast cells. LAT is highly phosphorylated following Fc ϵ RI engagement and its absence leads to a severe decrease in the activation and phosphorylation of its associated proteins, such as SLP-76, Vav, and PLC- γ 1/2. Consequently, mast cells derived from the bone marrow of these mice have impaired MAPK activation, cytokine production, and degranulation. The function of LAT in mast cells has also been demonstrated *in vivo*. Despite having normal numbers of mature mast cells, LAT^{-/-} mice are resistant to IgE-mediated passive systemic anaphylaxis, indicating the essential role of LAT in Fc ϵ RI-mediated signaling (5).

Unlike T and B cells, which seem to preferentially express either LAT or LAB, mast cells also highly express LAB. Upon Fc ϵ RI ligation, LAB is phosphorylated and interacts with Grb2. Interestingly, LAB phosphorylation is increased in LAT-deficient cells, perhaps in an attempt to compensate for the loss of LAT signaling. However, in contrast to LAT^{-/-} mast cells, LAB^{-/-} mast cells show enhanced degranulation. Fc ϵ RI-mediated PLC- γ phosphorylation, calcium mobilization, and Erk activation are also increased, implying a negative role for LAB in mast cell function. Yet, mast cells lacking both LAT and LAB

have a more dramatic block in FcεRI signaling than LAT^{-/-} mast cells, indicating that LAB may also exert a positive effect on FcεRI-mediated signaling (40, 49). Additionally, analyses using electron microscopy demonstrate that LAB and LAT localize to different microdomains in the plasma membrane before and after FcεRI activation. Thus, these two proteins seem to affect FcεRI-mediated signaling through autonomous mechanisms (50). In addition to its role in FcεRI-mediated signaling, LAB may also function in c-Kit-mediated signaling. Kit is able to directly phosphorylate LAB at different residues than Lyn and Syk. Various receptors may utilize LAB in different ways depending upon the signal being propagated, adding another dimension of flexibility in signaling through adaptor proteins (51).

LAT has been shown to negatively impact signaling through the recruitment of the phosphatase SHIP1 (52, 53). Upon deletion of LAB in mast cells, LAT-dependent positive as well as negative signaling events are enhanced, including the recruitment of SHIP1. SHIP1 is able to negatively regulate FcεRI signaling in two ways. First, it is able to remove phosphate groups from PIP₃, thus disrupting membrane recruitment of signaling molecules that contain a pleckstrin homology domain. Second, SHIP1 can recruit RasGAP and Dok-1 to quell Ras activity. Consequently, LAB^{-/-} mast cells have decreased Akt phosphorylation and survival due to the enhanced recruitment of SHIP1 by LAT (54). However, in contrast to these published results, our unpublished data show that Akt activation and cell survival are enhanced in LAB^{-/-} mast cells. It is not clear what causes these different observations.

IgE-induced mast cell survival is known to be dependent upon Syk activity and sustained Erk activation (55). Analysis of LAB^{-/-}LAT^{-/-} BMMCs shows that both of these adaptor proteins contribute to this process. Double-deficient cells have impaired survival, IL-3 induction, and Ras activation. However, these processes can be rescued upon expression of membrane-targeted Sos, which bypasses its recruitment by Grb2. Therefore, LAT and LAB are both critical for mast cell survival through their recruitment of Grb2 and activation of the Ras pathway (56).

LAT and LAB in NK cells

Natural killer cells express both LAT and LAB. Upon crosslinking of CD16 or engaging target cells, LAT is phosphorylated (57, 58); however, LAT^{-/-} NK cells are still able to lyse susceptible target cells and mediate ADCC (antibody-dependent cell-mediated cytotoxicity) (4). LAB is an obvious candidate that may compensate for the absence of LAT. Both proteins have been implicated in signaling through the NK receptor family Ly49D (59). A study comparing the roles of LAT and LAB in NK cell signaling shows that resting LAT^{-/-} NK cells have intact responses; however, activated NK cells from LAT^{-/-} mice have an impaired response to NK1.1, an activating ITAM-coupled receptor. LAB^{-/-} NK cells, on the other hand, have enhanced NK1.1 signaling upon activation, reminiscent of the negative role of LAB in FcεRI-mediated signaling. Moreover, the deletion of both of these proteins in resting and active NK cells causes a severe defect in NK1.1 signaling (60). Natural killer cells, then, are able to transmit signals using LAT and LAB and the interplay between these two can add plasticity to the NK response.

Future work in the field of adaptor proteins

Much progress has been made towards understanding how the LAT family of adaptor proteins function in the immune system, but there are still many issues to be resolved. Palmitoylation and phosphorylation are two critical post-translational modifications that can impact the localization and function of these adaptor proteins. The tyrosine kinases and sites of tyrosine phosphorylation of LAT and LAB have been well characterized. LAT is also heavily phosphorylated on Ser/Thr residues (unpublished observations). Another interesting

study indicates that Erk and Jnk are able to phosphorylate LAT at its Thr155 residue following TCR engagement, leading to a defect in the ability of LAT to recruit PLC- γ 1 and SLP-76, ultimately resulting in a decrease in calcium mobilization and MAP kinase activation (61). However, this threonine residue is only present in human LAT. Identification of other Ser/Thr sites, kinases and the functional importance of this type of phosphorylation will be crucial for a better understanding of the LAT family. In addition, it is very clear that palmitoylation of LAT and LAB is important for their function, but which acyl transferases are responsible for the palmitoylation of LAT and LAB are still unknown. Studies done in recent years indicate that a family of DHHC proteins that have a core cysteine rich domain (CRD) containing a sequence of Asp-His-His-Cys (DHHC) are the acyl transferases that can palmitoylate LAT or LAB (62). Which one (s) palmitoylates LAT or LAB and whether these enzymes are regulated during T cell activation or anergy induction are likely to be one of the most important issues to be addressed in future studies.

Another complex and critical issue that remains to be resolved is the role of LAT in the control of T cell homeostasis and autoimmunity. LATY136F mice, LAT3YF mice, and mice with LAT inducibly deleted all develop autoimmunity, although with different levels of severity. While our studies clearly indicate that LAT is important in T_{reg} function, the inability of these T_{reg} cells to suppress the proliferation of conventional T cells is certainly not the only reason contributing to autoimmunity. The signals generated from LAT that controls the expansion of conventional CD4⁺ T cells remain to be studied. Certainly, the negative role of LAT in controlling autoimmune responses and regulating T cell homeostasis may be just as critical as its role as a positive regulator of T cell signaling.

There are still many aspects of LAB-mediated signaling that remain unknown. While it is clear that LAB has a negative role in the regulation of T cell activation, how it accomplishes this is far from understood. Moreover, different from LAT, LAB is highly expressed in other hematopoietic cells, such as dendritic cells and macrophages. Studies of LAB in TREM-1- and TREM-2-mediated signaling suggest a potential role for LAB in the innate immune response, but whether LAB functions in innate and adaptive immune responses against different pathogens remains to be investigated. As the study of adaptor proteins continues to move forward, a more clear view of the intricate relationships involving LAT and LAB in modulating immune responses will be revealed.

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