The authors regret a minor error in Fig. 4B, which is corrected below. The error does not affect the conclusions of the article.

### Diagram Description

**B**

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**Thymocytes**

- **Anti-pY**
  - Lanes: 1, 2, 3, 4, 5, 6
  - Controls: medium, x(TCR+CD4), x(TCR+CD8)
  - Markers: pLAT, pPLCγ, pCbl

- **Anti-LAT**
  - Lanes: 7, 8, 9, 10, 11, 12
  - Controls: medium, x(TCR+CD4), x(TCR+CD8)
  - Markers: LAT
Association of the Adaptor Molecule LAT with CD4 and CD8 Coreceptors Identifies a New Coreceptor Function in T Cell Receptor Signal Transduction

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Summary

Linker for activation of T cells (LAT) is an adaptor protein whose tyrosine phosphorylation is critical for transduction of the T cell receptor (TCR) signal. LAT phosphorylation is accomplished by the protein tyrosine kinase ZAP-70, but it is not at all clear how LAT (which is not associated with the TCR) encounters ZAP-70 (which is bound to the TCR). Here we show that LAT associates with surface CD4 and CD8 coreceptors and that its association is promoted by the same coreceptor cysteine motif that mediates Lck binding. In fact, LAT competes with Lck for binding to individual coreceptor molecules but differs from Lck in its preferential association with CD8 rather than CD4 in CD4⁺CD8⁻ thymocytes. Importantly, as a consequence of LAT association with surface coreceptors, coengagement of the TCR with surface coreceptors induces LAT phosphorylation and the specific recruitment of downstream signaling mediators to coreceptor-associated LAT molecules. These results point to a new function for CD4 and CD8 coreceptors in TCR signal transduction, namely to promote LAT phosphorylation by ZAP-70 by recruiting LAT to major histocompatibility complex–engaged TCR complexes.

Key words: T cell receptor • development • signaling • thymus • phosphorylation

Activation of T lymphocytes in response to antigenic stimulation is initiated by interaction of surface TCRs with antigenic peptides bound to MHC molecules. Inside the cell, TCR engagement of MHC–peptide complexes results in activation of src family protein tyrosine kinases (PTKs) p56³k (Lck) and p59⁵k to phosphorylate specialized signaling motifs (referred to as immunoreceptor tyrosine-based activation motifs [ITAMs]) that are present in the cytosolic tails of the invariant components of surface TCR complexes (1–4). Phosphorylated ITAMs then recruit ZAP-70 to surface TCR complexes, whereupon the recruited ZAP-70 molecules are themselves tyrosine phosphorylated and activated (5–10). These proximal TCR signaling events are significantly enhanced by MHC-induced coengagement of TCR with surface CD4/CD8 coreceptor molecules, since such coengagement serves to juxtapose TCR complexes with coreceptor-associated Lck molecules and so promotes efficient phosphorylation of TCR ITAMs (11, 12) and efficient activation of TCR-associated ZAP-70 PTK molecules (13). Further propagation of the TCR signal requires tyrosine phosphorylation by ZAP-70 of linker for activation of T cells (LAT), a 36/38-kd membrane-associated protein that, upon tyrosine phosphorylation, is subsequently bound by downstream signaling mediators (14–16). Thus, tyrosine phosphorylation of LAT marks the transition between proximal and downstream signaling events initiated by TCR engagement of MHC–peptide complexes.

Because of LAT's central role in TCR signal transduction, it is important to understand how TCR-associated ZAP-70 PTK molecules encounter LAT in order to tyrosine phosphorylate it. In this study, we examined the possibility that LAT, like Lck, might be associated with CD4 and CD8 coreceptor molecules and so might be colocalized with TCR-associated ZAP-70 molecules upon MHC-induced coengagement of the TCR with CD4/CD8 coreceptors. We report that LAT does associate with CD4 and CD8 coreceptors in T cells, and that coengagement of TCR with surface coreceptor molecules induces LAT phosphorylation and recruitment of downstream signaling mediators to coreceptor-associated LAT molecules.

Abbreviations used in this paper: GEM, glycolipid-enriched membrane microdomain; ITAM, immunoreceptor tyrosine-activated motif; LAT, linker for activation of T cells; PI-3, phosphatidylinositol-3; PLC, phospholipase C; PTK, protein tyrosine kinase; SH2, src homology 2.
**Materials and Methods**

Cell Preparation and Immunoprecipitations. The care of experimental animals was in accordance with National Institutes of Health guidelines. Single cell suspensions of lymph node cells and thymocytes were prepared from C57BL/6 or CD8<sup>b</sup> mice thymi. Purified populations of CD<sup>4</sup>CD<sup>8</sup><sup>b</sup> thymocytes >96% pure were obtained by anti-CD8 panning of whole thymocyte populations and selecting the adherent cells, as previously described (17). Cells (0.5–3 × 10<sup>6</sup>, as indicated) were lysed in ice-cold lysing buffer containing either 1% Triton X-100 or 60 mM octylglucoside. After clarification (10,000 g for 10 min), cell lysates were subjected to immunoprecipitation with the indicated antibodies. Immunoprecipitates were resolved on SDS-PAGE under reducing conditions. Antibodies used for immunoprecipitation were specific for: CD4 (GK 1.5; PharMingen), CD8<sub>a</sub> (53-6-7; PharMingen), or CD8<sub>b</sub> (53-5-8; PharMingen); and TCR-ζ (serum 551, Lck (serum 688), or LAT (serum 3023) (14). Antibodies used for immunoblotting were specific for: LAT (serum 3023); Lck (serum 688); or phosphotyrosine (4G10; Upstate Biotechnology). For immunoblotting, mAbs were directly coupled to CNBr-activated Sepharose beads (Amersham Pharmacia Biotech), except when indicated otherwise.

Thymocyte Stimulation. Pervanadate treatment (final concentration of 0.01 mM Na<sub>2</sub>VO<sub>4</sub> in the presence of 4.5 mM H<sub>2</sub>O<sub>2</sub>, extemporaneously prepared) was conducted for 10 min at 37°C. TCR cross-linking experiments were performed essentially as described (13). Antibodies used for cross-linking were as follows: anti-TCR-β (H57-597 [18]), anti-CD4 (GK 1.5; PharMingen), and anti-CD8<sub>a</sub> (2.43 [19] or 53-6-7 [PharMingen]). Thymocytes were cultured for 18 h at 37°C, pelleted, and resuspended at 10<sup>7</sup>/ml in ice-cold RPMI containing 1 mM Na<sub>2</sub>VO<sub>4</sub> and biotinylated antibodies (10 μg/ml). After 10 min at 4°C, the cells were pelleted and resuspended at 10<sup>7</sup>/ml in RPMI containing 20 μg/ml of streptavidin (Southern Biotechnology Associates) previously prewarmed at 37°C. After a 5-min incubation at 37°C, cells were pelleted and lysed in octylglucoside-containing buffer.

**Results**

To determine if coreceptor-LAT complexes existed on unstimulated murine T cells and thymocytes, we immunoblotted anti-CD4 and anti-CD8 immunoprecipitates for LAT (Fig. 1 A, left). In lymph node T cells that express CD4 and CD8 coreceptors on separate cell populations, we found that LAT was associated with both CD4 and CD8 (Fig. 1 A, lanes 2 and 3). In immature CD4<sup>+</sup>CD8<sup>b</sup> thymocytes, which express both CD4 and CD8 on individual cells, we found that LAT associated with CD8 in signifi-

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**Figure 1.** Association of LAT with CD4 and CD8 coreceptors in mature T cells and immature thymocytes. (A) Mouse lymph node cells (3 × 10<sup>6</sup> cell equivalents/lane) or thymocytes (0.5 × 10<sup>6</sup> cell equivalents/lane) were detergent solubilized by 1% Triton X-100 (lanes 1–8) or 60 mM octylglucoside (OCT, lanes 9–13), and the resulting lysates were immunoprecipitated with either anti-CD4 or anti-CD8 antibodies directly coupled to beads. Unfractionated cell lysates (3–6 × 10<sup>6</sup> cell equivalents/lane) and immunoprecipitates (ip) were resolved by SDS-PAGE, immunoblotted with a LAT-specific rabbit antiserum, and visualized by enhanced chemiluminescence. In contrast to anti-CD4 and anti-CD8 immunoprecipitates, which contained LAT, control anti-ζ immunoprecipitates did not contain LAT (data not shown). As additional negative specificity controls, antibody-coupled beads in the absence of lysate were also immunoblotted for LAT (lanes 4 and 5). In lanes 10–13, anti-CD8<sub>a</sub> immunoprecipitates and unfractionated cell lysates from CD8<sup>b</sup> thymocytes were assayed for LAT and compared with wild-type CD8<sup>b</sup> thymocytes. (B) Mouse thymocytes were lysed in 60 mM octylglucoside, immunoprecipitated (ip) with anti-CD4 or anti-CD8 mAbs directly coupled to beads, and immunoblotted with either anti-Lck or anti-LAT. LAT was preferentially immunoprecipitated by CD8, whereas Lck was preferentially immunoprecipitated by CD4 (lanes 1 and 2). As an additional specificity control, neither LAT nor Lck was immunoprecipitated by beads in the absence of anti-CD4 or anti-CD8 antibodies (lane 3).
cantly greater amounts than with CD4 (Fig. 1 A, lanes 6 and 7). Interestingly, the hierarchy of LAT binding in immature CD4+CD8+ thymocytes (CD8 > CD4) is reciprocal to Lck, which binds CD4 > CD8 (24, 25; Fig. 1 B).

We then analyzed CD8–LAT associations in thymocytes in greater detail. LAT molecules are largely localized to detergent-resistant areas of the plasma membrane referred to as glycolipid-enriched membrane microdomains (GEMs) [22, 26], which also contain subpopulations of both CD4 and CD8 molecules (27). Thus, it was conceivable that coinmunoprecipitation of LAT with CD4/CD8 coreceptors might simply reflect the presence of LAT and CD4/CD8 coreceptors in the same GEM. This was not the case, however, as LAT–CD8 associations were maintained in anti-CD8 immunoprecipitations of cells solubilized by the detergent octylglucoside, which solubilizes GEMs (28, 29; Fig. 1 A, lanes 8 and 9). In fact, detergent solubilization with octylglucoside increased the amount of LAT detected in anti-CD8 immunoprecipitates (Fig. 1 A, lanes 8 and 9).

Next, we assessed whether LAT association with CD8 required both components of CD8 (α and β). In normal mice, CD8 is expressed on thymocytes and thymic-derived T cells as an αβ heterodimer, whereas in CD8β- mice, CD8 is expressed as an αα homodimer (30, 31). Despite similar amounts of LAT in whole cell lysates from normal and CD8β- thymocytes (Fig. 1 A, lanes 12 and 13), the amount of CD8-activated LAT was markedly greater in CD8β- thymocytes than in CD8β- thymocytes, although a small amount of LAT binding to CD8αα homodimers was evident in CD8β- thymocytes (Fig. 1 A, lanes 10 and 11). Thus, CD8β contributes to CD8–LAT association, as it does to CD8–Lck association (32, 33). For Lck, CD8β is thought to increase accessibility to a binding site present in the cytosolic tail of CD8α, and this may be the case for LAT as well.

To determine the molecular basis for LAT–coreceptor associations, we cotransfected 293T cells, a human transformed kidney cell line expressing SV40 T antigen, with murine LAT (mLAT) and murine coreceptor molecules (CD8α and β or CD4). The transfected coreceptor molecules were either wild-type or variants containing altered transmembrane or cytosolic domains (Fig. 2 A). Surface expression of transfected coreceptor molecules was quantitated by immunofluorescence and flow cytometry and was found to be similar within all experimental groups (Table 1). Lysates from transfected 293T cells were immunoprecipitated by anti-CD8α mAbs (Fig. 2 B, left) or anti-CD4 mAb (Fig. 2 B, right), and the immunoprecipitates were immunoblotted for LAT. Both CD8–LAT and CD4–LAT complexes were observed in 293T cells transfected with intact (AAA, 444) coreceptor molecules (Fig. 2 B, lanes 3 and 13), demonstrating that coreceptor–LAT complexes could form in nonlymphoid cells and did not require Lck, which is not expressed in 293T cells. We further documented that LAT did not associate with CD4 or CD8α variants expressing either the cytosolic tail of CD8β (lanes 4 and 12) or lacking a cytosolic tail altogether (lanes 5 and 9). Thus, the cytosolic tails of CD4 and CD8α are the main determinants for LAT association, suggesting that a sequence common to both cytosolic tails is involved in LAT binding.

Sequence homology between the cytosolic domains of CD4 and CD8α is restricted to a short region centered around a CxC cysteine motif that is involved in Lck binding (34, 35). To examine the possible role of this cytosolic CxC cysteine motif in LAT–coreceptor associations, we constructed a CD8α variant (AAM) in which both cytosolic cysteines C227 and C229 were mutated to alanines (Fig. 2 A). We found that substitution with alanine of these two cysteines diminished CD8α associations with murine LAT by 70% (Fig. 2 B, compare lanes 8 and 10). Thus, the cytosolic CxC cysteine motif important for coreceptor associations with Lck is also important for coreceptor associations with LAT.

Because LAT molecules contain two molecular species that migrate at either 36 or 38 kD in SDS-PAGE (14, 22), we wished to determine if coreceptors preferentially associated with one or the other form of LAT. To address this issue, we used human LAT (hLAT) because the 36- and 38-kD forms of hLAT are easily distinguishable (Fig. 2 C, lysate). We found that CD8 coreceptors were associated almost exclusively with the lower 36-kD form of LAT, and that that association was dependent on the cytosolic cysteines in the CD8α tail (Fig. 2 C, lanes 1–3). However, appearance of the lower 36-kD form of hLAT is dependent on two juxtamembrane cysteines (C26, C29) in the cytosolic tail of hLAT, which are palmitoylated and responsible for targeting LAT molecules to GEMs (22). Alanine substitution of both cysteines (C26 / 29A) removes the palmitoylation sites and results in migration of LAT as a single 38-kD (upper) band (Fig. 2 C, lanes 4 and 5). Interestingly, despite the fact that the C26 / 29A mutant LAT molecule cannot be palmitoylated and targeted to GEMs, we found that the mutant LAT can still associate with surface CD8 coreceptor molecules (Fig. 2 C, lanes 4 and 5). These results confirm that CD8–LAT associations are the result of specific protein–protein interactions and not simply of colocalization of both molecules in GEMs. However, we do not yet understand why CD8 normally preferentially associates with the lower band of LAT when it is also clearly capable of binding to the upper band.

Since the cytosolic cysteine motif in CD8α promotes binding of LAT as well as Lck, we considered that LAT and Lck might compete for binding to individual coreceptor molecules. To address this issue, we transiently expressed CD8α, CD8β, and mLAT in 293T cells. In addition, we also transfected the cells with an excess of either Lck-containing vector or empty vector (Fig. 3). Even though Lck did not affect the amount of LAT present in the cell lysates, Lck abrogated the ability of LAT to be immunoprecipitated by anti-CD8α (Fig. 3). Thus, Lck protein, when present in excess, competes with LAT for binding to CD8.

Since LAT function in TCR signal transduction depends on its tyrosine phosphorylation, we examined the ability of coreceptor-associated LAT molecules to be tyrosine phosphorylated. Treatment of thymocytes with pervanadate to induce activation of intracellular protein tyrosine kinases (36)
resulted in tyrosine phosphorylation of CD8-associated LAT and recruitment of LAT-binding phosphoproteins (Fig. 4 A) previously identified to include phospholipase C (PLC) -γ1 and Cbl, among others (14). Tyrosine phosphorylation of coreceptor-associated LAT molecules was also induced in thymocytes by antibody-mediated coengagement of TCR with either CD4 or CD8, but was not induced by TCR engagement alone (Fig. 4 B, lanes 1–6). Coengagement of TCR with either CD4 or CD8 also resulted in the appearance of phosphoprotein bands indicative of PLC-γ1 and Cbl (Fig. 4 B, lanes 7–12). Importantly, formation of oligomeric signaling complexes on coreceptor-associated LAT molecules occurred with a remarkable degree of specificity, preferentially forming on coreceptor-associated LAT molecules that had been coengaged with TCR, compared with LAT molecules that had not been coengaged with TCR. That is, PLC-γ1 and Cbl were preferentially recruited to CD4-associated LAT molecules by coengagement of TCR with CD4 compared with coengagement of TCR with CD8 (Fig. 4 B, lanes 7–9); and, reciprocally, PLC-γ1 and Cbl were preferentially recruited to CD8-associated LAT molecules by coengagement of TCR with CD8 compared with coengagement of TCR with CD4 (Fig. 4 B, lanes 10–12). The small amount of phosphorylated Cbl that was immunoprecipitated by antibodies specific for the nonengaged coreceptor probably reflects the passive and inadvertent capture of some nonengaged coreceptor molecules within the TCR aggregate (Fig. 4 B, lanes 9 and 11). Thus, corecep-
tors can promote LAT phosphorylation by TCR-associated ZAP-70 molecules and the subsequent recruitment of downstream signaling mediators.

**Discussion**

This study demonstrates that the LAT adaptor molecule associates with CD4 and CD8 surface coreceptors, and that such coreceptor associations are mutually exclusive with Lck. Indeed, the site on the cytosolic tail of CD4 and CD8α coreceptors to which LAT binds overlaps the site to which Lck binds, resulting in competition between Lck and LAT for coreceptor binding. Because of their association with coreceptor molecules, LAT molecules would be juxtaposed with TCR complexes upon coengagement of MHC–peptide complexes. In fact, we found that oligomeric complexes of downstream signaling mediators preferentially formed on those LAT molecules that were associated with coreceptors coengaged with the TCR. Thus, this study provides one solution to the problem of how TCR-associated ZAP-70 molecules can efficiently find their LAT substrate.

Colocalization of LAT with TCR would be a second function performed by CD4 and CD8 coreceptor molecules in TCR signal transduction, with colocalization of Lck and TCR being the only previously known function. In fact, we think that the two coreceptor functions are analogous to one another, in that coengagement of TCR with CD4 or CD8 coreceptor molecules by MHC–peptide complexes serves to physically juxtapose both Lck and LAT with TCR (schematized in Fig. 5). As a result, coreceptors promote both the initiation of TCR signaling and the activation of downstream signaling mediators. It is tempting to consider the implications of our present observations on the distinct but overlapping roles performed by CD4 and CD8 coreceptors in promoting TCR signal transduction in immature CD4+CD8+ thymocytes during T cell development in the thymus, as CD4 engagement by intrathymic MHC class II molecules would preferentially promote Lck activation (25), whereas CD8 engagement by intrathymic MHC class I molecules would preferentially promote LAT phosphorylation and activation of downstream mediators.

It is conceivable that such coreceptor-induced differences in TCR signal transduction pathways influence lineage choices, but this possibility has not yet been examined. Unfortunately, LAT knockout mice have not been informative for this issue, as the absence of LAT arrests thymocyte development at an early CD4+CD8− stage of development that precedes expression of CD4 and CD8 coreceptors as well as the point at which CD4/CD8 lineage determination occurs (37).

The association of LAT with surface CD4 and CD8 coreceptor molecules provides one solution to the problem of how TCR-associated ZAP-70 molecules manage to contact and phosphorylate LAT molecules. Indeed, we found that association of LAT with CD4 and CD8 coreceptors

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**Table I.** Coreceptor Expression in Transfected 293T Cells

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Surface expression of transfected coreceptor molecules on 293T kidney cells from Fig. 2 B was quantitated by surface staining with either anti-CD8α (experiment [Exp.] 1 and 2) or anti-CD4 (experiment 3) mAbs and flow cytometry. Surface staining of transfected cells was unimodal, and mean fluorescence intensities (expressed in fluorescence units) are indicated for each construct. Within each experiment, mean fluorescence intensity was normalized for each construct relative to that of the wild-type coreceptor construct (either AAA or 444), whose relative fluorescence intensity was set at 1.0.

*Sample numbers refer to lane numbers in Fig. 2 B.*

**Figure 3.** Overexpression of Lck inhibits CD8–LAT association in 293T cells. 293T cells were transfected with CD8α, CD8β, and mLAT, and either an Lck expression vector (10-fold excess over the mLAT vector, lane 1) or the same amount of empty control vector (lane 2). Expression of Lck did not detectably change either total LAT expression or surface CD8 expression (data not shown). However, overexpression of Lck resulted in CD8-Lck associations and in the inhibition of CD8-LAT associations. Relative fluorescence intensities (as defined in Table I) for anti-CD8α immunostaining of cells in lane 1 and 2 were identically 1.0.
was of functional significance for TCR signal transduction, as: (a) coreceptor-associated LAT molecules were tyrosine phosphorylated upon TCR–coreceptor coengagement, and, more importantly, (b) the scaffold for oligomerization of downstream signaling mediators preferentially formed on coreceptor-associated LAT molecules that were coengaged with TCR. Thus, coreceptor-induced colocalization of LAT with TCR promotes downstream TCR signaling events.

We found that coreceptor-LAT associations are promoted by the same dicysteine motif in the coreceptor tails that promotes Lck binding. Nevertheless, we do not think that Lck and LAT bind to coreceptor molecules through identical mechanisms. Association of coreceptor molecules with Lck involves formation of a zinc-dependent complex between the dicysteine motif in the coreceptor tail and two cysteines in Lck (38), and strictly requires those cysteines to be present in both the coreceptor tail and the Lck molecule (34, 35). In contrast, association of coreceptor molecules with LAT does not require cysteines in LAT, as CD8–LAT interactions were not abolished by mutation of LAT dicysteines to alanines. In fact, association of coreceptor molecules with LAT also does not strictly require the presence of the dicysteine motif in the coreceptor tail, as mutation of these coreceptor cysteines did not abrogate (but significantly reduced) association with LAT. Thus, we think that the molecular basis for LAT–coreceptor association is distinct from that of Lck–coreceptor association despite their overlapping binding sites. That Lck and LAT actually bind to overlapping sites in the cytosolic tail of CD8 is indicated by cotransfection experiments in which Lck was found to disrupt LAT binding to CD8. Such mutual exclusivity of binding may account for the opposite coreceptor binding preferences of LAT and Lck in CD4+CD8+ thymocytes, such that LAT may be primarily associated with CD8 because Lck is primarily associated with CD4. However, we favor the possibility that Lck and LAT have intrinsically different binding preferences for CD4 and CD8 coreceptor molecules such that Lck binds CD4 > CD8 and LAT binds CD8 > CD4.

LAT molecules are normally palmitoylated and, as a consequence, localized to GEMs (22). Importantly, however, LAT–coreceptor associations are mediated through protein–protein interactions and do not simply reflect their colocalization in GEMs, as LAT–coreceptor associations occur even in the absence of LAT palmitoylation. Indeed, a nonpalmitoylated version of LAT (C26/29A LAT) which...
does not localize in GEMs still associated with CD8. Nevertheless, it should be appreciated that subpopulations of CD4 and CD8 coreceptor molecules are normally present in GEMs (27), perhaps as a result of their association with palmitoylated LAT molecules. Consistent with such a possibility, we found that detection of CD8-LAT complexes was significantly increased by solubilization of cells with the detergent octylglucoside, which solubilizes GEMs far better than the detergent Triton X-100.

Surface CD4/CD8 coreceptors are not strictly required for TCR signal transduction, as CD4–CD8+ T cells, in the absence of either CD4 or CD8 coreceptor molecules, competently transduce TCR signals. Consequently, there must exist coreceptor-independent mechanisms for promoting LAT phosphorylation by ZAP-70, although they may be less efficient than the physical colocalization resulting from LAT association with surface coreceptor molecules. It is conceivable that trapping of GEM-associated LAT molecules between aggregated TCR complexes can induce LAT phosphorylation. Such a possibility is suggested by the finding that TCR stimulation results in the migration of TCR components into GEMs (39, 40). Importantly, however, it is not at all clear that ZAP-70 actually translocates to GEMs, a necessary event for ZAP-70 phosphorylation of GEM-associated LAT, as conflicting results have been reported (22, 39, 40). In any event, the inadvertent trapping of LAT between TCR aggregates would be expected to be significantly less efficient than the juxtaposition of LAT and TCR induced by coengagement of MHC-peptide complexes.

It has been suggested that LAT phosphorylation by ZAP-70 may be enhanced by proteins containing Src homology 2 (SH2) domains such as 3BP2, phosphatidylinositol-3 (PI-3) kinase p85 regulatory subunit, or PLC-γ1. 3BP2 is an SH2-containing adaptor protein that binds to tyrosine-phosphorylated forms of LAT and ZAP-70 (41). Because 3BP2 contains only one SH2 domain, it is not evident how it could couple activated ZAP-70 to LAT. PI-3 kinase p85 subunit is a protein that contains two SH2 domains and has been shown in platelets to couple tyrosine-phosphorylated LAT with other tyrosine-phosphorylated molecules such as FcR-γ chain (42). Similarly, PLC-γ also has two SH2 domains and could conceivably couple ZAP-70 to LAT (43). However, because SH2 domain-containing linker proteins only bind tyrosine-phosphorylated substrates, they would be expected to promote ZAP-70 associations with already phosphorylated LAT molecules, they would not be expected to promote LAT's initial phosphorylation by ZAP-70. Consequently, the molecular basis for the initial phosphorylation of LAT by ZAP-70 in the absence of CD4 and CD8 coreceptors remains uncertain.

In conclusion, this study documents the association of LAT with CD4 and CD8 coreceptor molecules in resting T cells and thymocytes, and documents that coengagement of coreceptor molecules with surface TCR results in tyrosine phosphorylation of LAT and recruitment of downstream signaling mediators.

Figure 5. Model for the role of coreceptor-associated molecules in TCR signal transduction. Coengagement of TCR and CD4/CD8 coreceptor molecules by MHC-peptide complexes induces the physical approximation of coreceptor-associated Lck molecules within TCR aggregates, resulting in efficient tyrosine phosphorylation of TCR ITAMs, recruitment of ZAP-70, and efficient tyrosine phosphorylation of TCR-associated LAT. Coreceptor-associated LAT molecules are then bound via SH2 domain interactions by downstream mediators of TCR signal transduction. In this model, CD4/CD8 coreceptors perform two important functions in TCR signal transduction: colocalization of TCR with Lck, and colocalization of TCR with LAT. We think that these two coreceptor functions are mediated by individual coreceptor molecules that are associated either with Lck or with LAT, but alternative stoichiometries are possible.
References


