Functional Uncoupling of T-cell Receptor Engagement and Lck Activation in Anergic Human Thymic CD4+ T Cells*

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Human thymic CD1a-CD4+ T cells in the final stage of thymic maturation are susceptible to anergy induced by a superantigen, toxic shock syndrome toxin-1 (TSST-1). Thymic CD4+ T-cell blasts, established by stimulating human thymic CD1a-CD4+ T cells with TSST-1 in vitro, produce a low level of interleukin-2 after restimulation with TSST-1, whereas TSST-1-induced adult peripheral blood (APB) CD4+ T-cell blasts produce high levels of interleukin-2. The extent of tyrosine phosphorylation of the T-cell receptor ζ chain induced after restimulation with TSST-1 was 2–4-fold higher in APB CD4+ T-cell blasts than in thymic CD4+ T-cell blasts. The tyrosine kinase activity of Lck was low in both thymic and APB CD4+ T-cell blasts before restimulation with TSST-1. After restimulation, the Lck kinase activity increased in APB CD4+ T-cell blasts but not in thymic CD4+ T-cell blasts. Surprisingly, Lck was highly tyrosine-phosphorylated in both thymic and APB CD4+ T-cell blasts before restimulation with TSST-1. After restimulation, it was markedly dephosphorylated in APB CD4+ T-cell blasts but not in thymic CD4+ T-cell blasts. Lck from APB CD4+ T-cell blasts bound the peptide containing the phosphotyrosine at the negative regulatory site of Lck-505 indicating that the site of dephosphorylation in TSST-1-activated T-cell blasts is Tyr-505. Confocal microscopy demonstrated that colocalization of Lck and CD45 was induced after restimulation with TSST-1 in APB CD4+ T-cell blasts but not in thymic CD4+ T-cell blasts. Further, remarkable accumulation of Lck in the membrane raft was observed in restimulated APB CD4+ T-cell blasts but not in thymic CD4+ T-cell blasts. These data indicate that interaction between Lck and CD45 is suppressed physically in thymic CD4+ T-cell blasts and plays a critical role in sustaining an anergic state.

Human thymic CD1a-CD4+ T cells in the final stage of maturation (1) and cord blood CD4+ T cells are susceptible to anergy induction by in vitro stimulation with a superantigen, toxic shock syndrome toxin-1 (TSST-1). Thymic and cord blood CD4+ T-cell blasts, prepared by stimulating human thymic CD1a-CD4+ and cord blood CD4+ T cells with TSST-1, exhibited low IL-2, IL-4, and interferon-γ production and reduced proliferation after restimulation with TSST-1, whereas adult peripheral blood (APB) CD4+ T-cell blasts prepared in the same way exhibited high responses (2, 3). These characteristics indicate that cord blood and thymic CD4+ T cells are still functionally immature and that a post-thymic maturation process occurs in human peripheral blood T cells. To understand why these cells respond differently, it is essential to analyze the signal transduction pathway via T-cell receptor stimulation in anergic TSST-1-induced thymic CD4+ T-cell blasts.

Signal transduction via TCR stimulation involves at least two pathways: (i) activation of phospholipase C-γ1 and (ii) activation of the Ras-MAPK (mitogen-activated protein kinase) pathway. Both pathways require the activation of Src family kinases such as Lck and Fyn. Although mature T cells are highly resistant to anergy induction, they are rendered anergic when stimulated through TCR in the absence of costimulatory signals such as the CD28 molecule (4). Anergic T cells display various alterations in the two above mentioned pathways, including failure of ZAP-70, Ras, ERK (extracellular signal-regulated kinase), JNK (c-Jun NH2-terminal kinase), or AP-1 activation after restimulation with antigens (5–9). They also display constitutively elevated concentrations of intracellular free calcium and inositolphosphate, as well as increased tyrosine phosphorylation of phospholipase C-γ1, Fyn, and Bcl (10–12). It is not yet clear whether these defects are involved in the anergic state induced by TSST-1 in human thymic CD4+ T cells.

In the present study, we addressed the question of why human thymic CD1a-CD4+ T cells are induced into an anergic state after stimulation with TSST-1 by comparing signal transduction in anergic TSST-1-induced thymic CD4+ T-cell blasts with that in highly responsive TSST-1-induced APB CD4+ T-cell blasts. The results strongly suggest that the absence of an interaction between Lck and CD45 (which possesses phosphatase activity) is responsible for maintaining the anergic state of human thymic CD1a-CD4+ T cells induced by TSST-1.

**EXPERIMENTAL PROCEDURES**

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¶ The abbreviations used are: TSST-1, toxic shock syndrome toxin-1; IL-2, interleukin-2; APB, adult peripheral blood; TCR, T-cell receptor; Ab, antibody; FITC, fluorescein isothiocyanate; APC, antigen-presenting cell; PBS, phosphate-buffered saline.

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TABLE I

| Phenytype | Before or after blast formation | Expression | Exp. CD4+ T-cell blasts | Stimulation period (h) | IL-2 production | units/ml |
|-----------|--------------------------------|------------|------------------------|------------------------|----------------|---------|
| Thymic T cells | | | | | | |
| CD3+ | After | 87.3 ± 7.8 | 68.8 ± 1.7 | 1 | APB | 129 | 2355 | 2290 |
| Vβ2+ | After | 98.5 ± 1.0 | 98.0 ± 1.3 | 2 | APB | 31 | 40.2 | 283 |
| CD4+ | Before | 81.6 ± 3.5 | 60.4 ± 4.2 | | | |
| After | 90.2 ± 6.9 | 95.3 ± 3.2 | 3 | APB | 48 | 788 | 818 |
| CD28+ | Before | 63.4 ± 1.4 | 68.9 ± 7.6 | 4 | APB | 133 | 1530 | 978 |
| After | 90.5 ± 8.2 | 94.4 ± 3.3 | | | |
| CD45RO | After | 71.8 ± 12.6 | 72.9 ± 16.5 | | | |
| HLA DR | After | 23.5 ± 8.8 | 65.2 ± 6.4 | | | |

The sequence of a tyrosine-phosphorylated Lck carboxyl-terminal peptide (LckP peptide) was synthesized by BEX (Tokyo, Japan). Precipitation of LckP peptide was done as described previously (15). Biotin-conjugated LckP peptide was used as antigen-presenting cell (APC) (15). Biotin-conjugated LckP peptide was used as antigen-presenting cell (APC) (15). Biotin-conjugated LckP peptide was used as antigen-presenting cell (APC) (15).

(anti-CD4), phycoerythrin-conjugated SK1 (anti-CD8) and FITC- or phycoerythrin-conjugated SK7 (anti-CD3, Becton Dickinson, Mountain View, CA); RD-1-conjugated I2 (anti-DR, Coulter Immunology, Hialeah, FL); biotin-conjugated E22E7.2 (anti-Vβ2) and FITC-conjugated UCHL.1 (anti-CD45RO, Immunotech, Marseille, France); FITC-conjugated anti-CD28 (Immunotech, Marseille, France); FITC-conjugated avidin (Vector Laboratories, Burlingame, CA); 4G10 (anti-phosphotyrosine, Upstate Biotechnology Inc., Lake Placid, NY); 6B10.2 (anti-TCRγδ and anti-CD45 (M-20; Santa Cruz Biotechnology Inc., Santa Cruz, CA); and FITC-conjugated anti-CD45 and Texas red-conjugated avidin (PharMingen, San Diego, CA). The anti-Lck Ab was kindly provided by Dr. T. Koga (Tokai University, Japan).

Preparation of APB CD4+ and Thymic CD1a+ CD4+ T-Cells—The procedures for preparing human lymphoid cells have been described previously (2). Briefly, APB mononuclear cells were isolated from the peripheral blood of healthy adult donors by Ficol-Conray density gradient centrifugation. Whole APB T cells were obtained by the sheep red blood cell rosette method. After obtaining written informed consent, single-cell suspensions of thymocytes were obtained from these fragments dissected from donors, ranging in age from 3 to 24 years, during corrective cardiac surgery. To obtain APB CD4+ T cells, whole APB T cells were treated with Abs Nu Ts/c and I2C3. To obtain thymic CD1a+ CD4+ T cells, thymocytes were first treated with peanut agglutinin, and then non-aggregated T cells were treated with a combination of Abs Nu Ts/c, I2C3, and OKT6. After washing, these preparations were subjected to Percoll density gradient centrifugation. The large lymphoblasts obtained at the interface of the culture medium and Percoll were expanded by incubation with 100 units/ml recombinant IL-2 for 4 days.

Assay for Production of IL-2—For the assay of IL-2, T-cell blasts (5 × 10^6 cells/well) were stimulated with 10 ng/ml TSST-1 on an APC monolayer for 3 days on an APC monolayer and then expanding the large lymphoblasts with recombinant IL-2 for 4 days.

The immunologic phenotypes of the CD4+ T cells were analyzed before and after blast formation with TSST-1.

Flow Cytometric Analysis—For expression of CD4 versus CD3, CD3 versus Vβ2, CD3 versus CD28, CD5 versus CD45RO, and CD3 versus HLA-DR in the T-cell preparations, T cells were stained with several combinations of the appropriate phycoerythrin-, FITC-, and FITC-conjugated Abs and examined by two-color flow cytometric analysis using an EPICS C8 flow cytometer (Coulter Electronics, Hialeah, FL) as described previously (3). All procedures for cell staining were conducted on ice.

Immunoprecipitation and Immunoblotting—T-cell blasts (2 × 10^7 cells/well) were restimulated with 2 μg/ml TSST-1 at 37 °C in 1-ml volumes in 6-well culture plates containing tightly adherent APC. At different time points, cultures were terminated by adding 5 ml of cold PBS, and T-cell blasts were collected immediately. After washing with cold PBS, the T-cell blasts were lysed in cold TNE buffer containing 10 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 1 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 0.05% sodium deoxycholate and 0.1% SDS) followed by washing buffer containing 20 mM Tris-HCl (pH 7.4) and 0.5% LiCl. Immunoprecipitates were incubated with 30 μl of kinase reaction buffer containing 40 mM HEPES (pH 7.4), 3 mM MnCl2, 10% glycerol, 10 μM cold ATP, and 10 μM of γ-32P-ATP (PerkinElmer Life Sciences) per sample. The reaction was allowed to proceed for 10 min at 30 °C, the samples were then centrifuged, and the supernatants were separated by reducing 8% SDS-polyacrylamide gel electrophoresis. After drying, the radioactivity bound to the gels was analyzed with a Molecular Imager System FX (Bio-Rad).

Precipitation of Tyrosine-phosphorylated Lck Carboxy-terminal Peptide—The sequence of a tyrosine-phosphorylated Lck carboxy-terminal peptide (LckP peptide) containing the carboxy-terminal 11 amino acids of Lck, Thr-Ala-Thr-Glu-Gly-Gln-Tyr-(PO3)3-Gln-Pro-Gln-Pro, has been described by Sieh et al. (15). Biotin-conjugated LckP peptide was synthesized by BEX (Tokyo, Japan). Precipitation of LckP peptide was done as described previously (15). Briefly, cell lysates from T-cell blasts stimulated as described in immunoprecipitation were incubated with 50 μg/ml biotinylated LckP peptide for 2 h at 4 °C followed by incubation with streptavidin-conjugated agrose beads (Sigma) for 2 h. The pre-
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were mounted using a glycerol solution containing anti-fade reagent (Molecular Probes, Eugene, OR).

Confocal imaging was performed with a laser head (MRC-600, Bio-Rad). Samples were excited at 488 and 568 nm, and fluorescein and Texas red signals were detected through the R2 and K1 filter blocks, respectively. Images were collected using the photon counting mode of the COMOS program (Bio-Rad). In double-labeling experiments, bleed-through of the Texas red signal into the fluorescein channel was negligible.

Sucrose Gradient Centrifugation—To obtain the raft membrane fraction, T-cell blasts (7.5 × 10^7) were lysed with 1 ml of cold TNE buffer containing 0.5% TX-100, 1% TSST-1, 0.15% NaCl, 1 mM EDTA, 1 mM NaN_3, 10 μg/ml aprotinin. The lysates were centrifuged for 5 min at 1300 × g to remove the nuclei and large cellular debris. For equilibrium centrifugation of the TX-100 lysates, the lysates were diluted with an equal volume of 80% w/v sucrose in TNE buffer. Then 0.8 ml of lysate-sucrose mixture was overlaid sequentially with 2 ml of 30% sucrose and 1 ml of 4% sucrose prepared in TNE, and the mixture was centrifuged at 200,000 × g for 16 h at 4°C in an RPS 50–2 rotor (Hitachi, Tokyo, Japan). The gradient was fractionated into 0.5-ml fractions from the top of the tube. The raft and TX-100-soluble fractions were obtained in fractions 1–3 and 6–7, respectively.

RESULTS

**TSST-1-induced Human Thymic CD4+ T-cell Blasts Are in an Anergic State**—A summary of the surface phenotypes of TSST-1-induced thymic and APB CD4+ T-cell blasts used in this study is presented in Table I (under "Immunologic phenotypes"). Both type of cells show the phenotypes of TSST-1-reactive T cells. The percentage of TCR Vβ2+ T cells, which are the major TSST-1-reactive human T-cell fraction (16), was around 80% in both the thymic and APB T-cell preparations. The intensity of TCR expression was equivalent in both preparations (data not shown). Most of the Vβ2-negative T cells among the thymic and APB T-cell blasts were likely to be other TSST-1-reactive fractions such as Vβ4+ T cells (17). The percentage of CD28+ T cells increased from around 70% before to around 90% after blast formation in both the thymic and APB T-cell preparations, and the percentage of CD45RO+ T cells increased to a similar extent among both types of T-cell blasts. It should be noted that the percentage of HLA DR+ T cells was 3-fold lower among the thymic CD4+ T-cell blasts than among the APB CD4+ T-cell blasts. The APB and thymic CD4+ T-cell blasts were then restimulated with TSST-1 (10 ng/ml) on an APC monolayer and examined for IL-2 production (Table I, under "IL-2 production"). IL-2 production was much lower in the thymic CD4+ T-cell blasts than in the APB CD4+ T-cell blasts; the amount of IL-2 produced by the former was at most 10% of that produced by the latter. However, both the thymic and APB CD4+ T-cell blasts exhibited substantial IL-2 production upon stimulation with a combination of PMA and ionomycin (data not shown). These results indicate that thymic CD4+ T-cell blasts are in an anergic state following exposure to TSST-1.

**Reduced Tyrosine Phosphorylation of the TCRζ Chain in Thymic CD4+ T-cell Blasts**—When T cells are activated by antigens, tyrosine phosphorylation of the TCRζ chain is induced (18). The extent of tyrosine phosphorylation of the TCRζ chain was therefore compared between TSST-1-induced thymic and APB CD4+ T-cell blasts. T-cell blasts were restimulated with TSST-1 (2 μg/ml) on an APC monolayer and cell lysates were prepared. The ζ chain was immunoprecipitated and the extent of tyrosine phosphorylation was analysed by Western blotting with an anti-phosphotyrosine Ab. As shown in the upper part of Fig. 1A, tyrosine phosphorylation of the ζ chain was minimal in the absence of TSST-1 restimulation but showed significant increase after restimulation in both the thymic and APB T-cell blasts. The extent of phosphorylation was 2- to 4-fold higher in APB CD4+ T-cell blasts than in thymic CD4+ T-cell blasts. No difference in expression of the ζ chain was detected between the thymic and APB T-cell blasts.
pictures are shown in the combined fluorescence. Bright field were observed 20 min after restimulation (data not shown).

Because up-regulation of the tyrosine kinase activity of Lck plays a critical role in T-cell activation through TCR (19), the kinase activity of the Lck present in TSST-1-induced thymic and APB CD4^+ T-cell blasts was studied next. Lck immunoprecipitates were prepared from restimulated T-cell blasts and were subjected to an in vitro kinase assay in which autophosphorylation of Lck was measured. In three independent experiments, a 2–14-fold increase in autophosphorylation was observed in the APB CD4^+ T-cell blasts after restimulation with TSST-1 (Fig. 1B). In contrast, no elevation in autophosphorylation was observed in the thymic CD4^+ T-cell blasts (Fig. 1B). These results indicate that the Lck kinase activity of APB CD4^+ T-cell blasts increases markedly upon restimulation with TSST-1, whereas no such increase is induced in thymic CD4^+ T-cell blasts.

Tyrosine kinase activity of src family kinases is regulated by phosphorylation and dephosphorylation of two tyrosine residues (Tyr-394 and Tyr-505 in Lck) (20–22). To determine why thymic CD4^+ T-cell blasts restimulated with TSST-1 showed only weak Lck kinase activity, the extent of Lck tyrosine phosphorylation was compared in thymic and APB CD4^+ T-cell blasts. Lck was immunoprecipitated from cell lysates of these T-cell blasts after restimulation with TSST-1 on an APC monolayer and analyzed by Western blotting using an anti-Lck Ab. As shown in Fig. 1, a 2–14-fold increase in autophosphorylated Tyr-505 of Lck was detected in APB CD4^+ T-cell blasts restimulated with TSST-1 on an APC monolayer for 10 min. The T-cell blasts were fixed, permeabilized, and double-stained with anti-Lck Ab (red) and anti-CD45 Ab (green). The upper two rows show the fluorescence from each antibody, and the third row shows the combined fluorescence. Bright field pictures are shown in the bottom row.

Up-regulation of the Lck kinase activity is associated with phosphorylation of Tyr-394 (the autophosphorylation site) and dephosphorylation of the carboxyl-terminal tyrosine (Tyr-505) (20–22). The results shown in Fig. 1, B and C, suggest that TSST-1 restimulation induced dephosphorylation of phosphorylated Tyr-505 in APB CD4^+ T-cell blasts but not in thymic CD4^+ T-cell blasts. Phosphorylated Tyr-505 of Lck has been shown to bind to its own Src homology-2 domain, and it is postulated that this binding can cause the molecule to fold and thereby block kinase activity (23, 24). When phosphorylated Tyr-505 is dephosphorylated, Lck “opens out” and recovers the capacity to interact with a tyrosine-phosphorylated Lck carboxyl-terminal peptide (LckP peptide) (15).

To determine whether the Tyr-505 residue of Lck is the site at which dephosphorylation induced by restimulation with TSST-1 occurs in APB CD4^+ T-cell blasts, binding of dephosphorylated Lck to LckP peptide was examined. Cell lysates prepared from T-cell blasts restimulated with TSST-1 on an APC monolayer were incubated with biotinylated LckP peptide. The precipitates were isolated with avidin-conjugated agarose beads and analyzed by Western blotting using an anti-Lck Ab. As shown in Fig. 1D (upper panels), the extent of precipitated Lck was minimal in the absence of TSST-1 restimulation, but restimulation in APB CD4^+ T-cell blasts significantly increased the binding of Lck. In contrast, the LckP peptide-bound Lck did not increase after restimulation and rather showed a slight decrease in thymic CD4^+ T-cell blasts. No difference in expression of the Lck was detected between the two T-cell populations (Fig. 1D, lower panels). These results show that the dephosphorylated Tyr-505 of Lck in TSST-1-induced APB CD4^+ T-cell blasts is in the “open” configuration, whereas Lck of thymic blasts is in a “closed” form.

Lck and CD45 Are Physically Uncoupled in Thymic CD4^+ T-cell Blasts—Because the Tyr-505 site of Lck is a potential substrate for the protein tyrosine phosphatase CD45 (25), CD45 may play a major role in the dephosphorylation of Lck in...
A T-cell blasts after restimulation with TSST-1. To determine how Lck dephosphorylation is suppressed in thymic T-cell blasts, the subcellular localization of CD45 and Lck was determined by confocal microscopy. T-cell blasts restimulated with TSST-1 on an APC monolayer were separated from the monolayer and stained with biotinylated anti-Lck Ab followed by Texas red-conjugated avidin. If Lck (red) and CD45 (green) were present in close proximity, the appearance of yellow dots would be observed. As shown in Fig. 2, Lck was expressed in both the membrane and cytoplasmic areas of unstimulated thymic and APB CD4 T-cell blasts, whereas CD45 was expressed mainly in the membrane. No colocalization of Lck and CD45 was observed in these cells. After restimulation with TSST-1, the intensity of the yellow color increased in the membrane of the APB CD4+ T-cell blasts, predominantly on one side of the blast, indicating that Lck and CD45 had become colocalized in the plasma membrane region after restimulation with TSST-1. In contrast, no colocalization of these two molecules was induced by restimulation of thymic CD4+ T-cell blasts. Although the distributions of Lck and CD45 are comparable with that of APB CD4+ T-cell blasts, double staining with anti-CD45 and anti-Lck clearly shows these molecules are present in discrete areas on the cell membrane.

Loss of Lck Localization into the Membrane Raft in Thymic T-Cell Blasts—Recent studies have shown that sphingolipid-and cholesterol-rich plasma membrane microdomains, termed membrane rafts, play an important role in TCR signal transduction. Raft aggregation promotes recruitment of signaling proteins such as Lck but excludes the tyrosine phosphatase CD45 (26, 27). Our data from confocal microscopy suggests that the physical interaction between CD45 and Lck in thymic T-cell blasts is much less than in APB T-cell blasts.

To test whether membrane rafts play a role in the process of Lck activation, we next determined the localization of Lck and CD45 inside and outside of the raft fraction. Cell lysates prepared from T-cell blasts restimulated with TSST-1 on an APC monolayer were lysed in a buffer containing nonionic detergent, and the lysates were fractionated by sucrose gradient centrifugation. As shown in Fig. 3A, Lck was mostly present outside of rafts in unstimulated T-cell blasts. After restimulation with TSST-1, a significant increase of Lck in rafts was observed in APB CD4+ T-cell blasts. In contrast, no increase of Lck in membrane rafts was induced by restimulation of thymic CD4+ T-cell blasts. This increase of Lck in the raft was more striking after a 70-min restimulation of APB T-cell blasts. Even at that point, no increase was observed in thymic T-cell blasts. Immunoprecipitation of Lck from the same fractionated lysates confirmed these results (Fig. 3A). In contrast to this accumulation of Lck in the raft, CD45 was present outside of the raft fraction throughout the restimulation in both types of T-cell blasts as previously reported (Fig. 3C).

DISCUSSION

Human thymic CD1a+CD4+ T cells are highly susceptible to anergy induction by superantigens, whereas APB CD4+ T cells are resistant, as shown in a previous study (2) and in Table I. T cells are rendered anergic when they are stimulated through TCR in the absence of costimulatory signals such as the CD28 molecule (4). However, the absence of a costimulatory signal is not responsible for the high susceptibility of thymic CD1a+CD4+ T cells to TSST-1-induced anergy, because the percentage of CD28+ T cells among unstimulated thymic and APB CD4+ T cells was similar. We did not find any differences in surface phenotypes to explain the differences in susceptibility to anergy induction between unstimulated thymic and APB CD4+ T cells (Table I). In the present study, we examined...
signal transduction in thymic and APB CD4+ T-cell blasts before and after restimulation with TSST-1. The results strongly suggest the presence of a novel regulatory mechanism that controls an interaction between Lck and CD45.

The activity of CD45 is regulated by several mechanisms including isoform specific regulation (28). In this study, the level of CD45RO expression was similar in both thymic and APB CD4+ T-cell blasts (Table I), ruling out the possibility that isoform differences caused the impairment of CD45 function in thymic CD4+ T-cell blasts. Instead, our results show a remarkable difference in the physical localization of CD45 and Lck after restimulation with TSST-1 in thymic CD4+ T-cell blasts. An analysis of the subcellular localization of CD45 and Lck in thymic and APB CD4+ T-cell blasts after restimulation with TSST-1 showed that Lck and CD45 were physically associated in the membrane region of APB CD4+ T-cell blasts but not thymic CD4+ T-cell blasts (Fig. 2).

Membrane-associated molecules are known to localize to a specifically designated area composed of discrete lipid microdomains (26, 27). Some but not all of the Lck localizes in sphingolipid-cholesterol rafts, whereas no association of CD45 with these rafts has been observed (27, 29). Several studies have shown that a synaptic structure is formed by dynamic relocalization of various molecules at the site of the interaction between T cells and APC (30–32). The central zone of the synaptic structure formed between T cells and APC is rich in TCR, CD4, and CD8 (30–32). The central zone of the synaptic structure formed between T cells and APC is rich in TCR, CD4, and CD8, whereas CD45 remains on the outside (31, 32). This structure formed between T cells and APC is rich in TCR, CD4, and CD28, whereas CD45 remains on the outside (31, 32). This view, Lck, which is regulated by interaction with CD45. In this view, Lck, which resides outside of the raft, gets dephosphorylated after stimulation and localizes in the raft. With thymic T-cell blasts, the outside to the inside of sphingolipid-cholesterol rafts is a dynamic process involved in the interaction between these two molecules. A potential model is that repartitioning of Lck from the outside to the inside of sphingolipid-cholesterol rafts is regulated by interaction with CD45. In this view, Lck, which resides outside of the raft, gets dephosphorylated after stimulation and localizes in the raft. With thymic T-cell blasts, the interaction between CD45 and Lck is blocked, and thus this translocation into the raft is blocked (Fig. 4, MODEL 1). Alternatively, although CD45 is not within the raft, it only interacts with Lck within the raft. In thymic T-cell blasts, Lck localization into rafts is blocked, and as a result, its interaction with CD45 is impaired (Fig. 4, MODEL 2). We are currently testing these hypotheses.

Taken together, these results suggest the presence of a novel regulatory mechanism that controls localization of CD45 and Lck in activated T cells. Most importantly, this regulation may play a critical role in the maintenance of the anergic state of human thymic CD1a−CD4+ T cells induced by superantigens.

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