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Essential Role of CD8 Palmitoylation in CD8 Coreceptor Function[1]

Alexandre Arcaro,* Claude Grégoire,† Nicole Boucheron,* Sabine Stotz,‡ Ed Palmer,‡ Bernard Malissen,† and Immanuel F. Luescher[2]*

To investigate the molecular basis that makes heterodimeric CD8$\alpha\beta$ a more efficient coreceptor than homodimeric CD8$\alpha\alpha$, we used various CD8 transfectants of T1.4 T cell hybridomas, which are specific for H-2K^d, and a photoreactive derivative of the *Plasmodium berghei* circumsporozoite peptide PbCS 252–260 (SYIPSAEKI). We demonstrate that CD8 is palmitoylated at the cytoplasmic tail of CD8β and that this allows partitioning of CD8$\alpha\beta$, but not of CD8$\alpha\alpha$, in lipid rafts. Localization of CD8 in rafts is crucial for its coreceptor function. First, association of CD8 with the src kinase p56^{lck} takes place nearly exclusively in rafts, mainly due to increased concentration of both components in this compartment. Deletion of the cytoplasmic domain of CD8β abrogated localization of CD8 in rafts and association with p56^{lck}. Second, CD8-mediated cross-linking of p56^{lck} by multimeric K^d-peptide complexes or by anti-CD8 Ab results in p56^{lck} activation in rafts, from which the abundant phosphatase CD45 is excluded. Third, CD8-associated activated p56^{lck} phosphorylates CD3ζ in rafts and hence induces TCR signaling and T cell activation. This study shows that palmitoylation of CD8β is required for efficient CD8 coreceptor function, mainly because it dramatically increases CD8 association with p56^{lck} and CD8-mediated activation of p56^{lck} in lipid rafts. *The Journal of Immunology,* **2000, 165: 2068–2076.**

CD8 on thymocytes and thymus-derived T cells consists of disulfide-linked CD8α and CD8β chains, which are transmembrane proteins, containing N-terminal Ig domains, extended and glycosylated hinge and stalk regions, and transmembrane and cytoplasmic portions (1, 2). In contrast, NK cells or intestinal T cells express homodimeric CD8, composed of disulfide-linked CD8α (1, 2). The Ig domain of CD8 interacts with the constant domain of MHC class I molecules (1, 2), and the cytoplasmic tail of CD8α can associate with the src kinase p56^{lck} (lck),[3] by means of a zinc cation, chelating vicinal cysteines on both molecules (3, 4). Therefore, this association is disrupted by EDTA or iodoacetamide. The 19-residue-long cytoplasmic tail of CD8β is not known to interact with other molecules but has been reported to increase CD8 association with lck (5, 6).

Several reports have shown that CD8$\alpha\beta$ is a much more effective coreceptor than CD8$\alpha\alpha$. Cells expressing CD8$\alpha\beta$ are able to recognize Ag at considerably lower concentrations than cells expressing CD8$\alpha\alpha$ (7). This difference is particularly striking for low affinity altered peptide ligands. Moreover, disruption of the CD8β gene or deletion of the cytoplasmic tail of CD8β results in severe reduction of positive selection of CD8$^+$ T cells (8–11). The molecular basis for these dramatic differences, in particular the role of CD8β in CD8 coreceptor function, remains enigmatic. Using TCR photoaffinity labeling with soluble monomeric K^d-peptide complexes, we have shown that on cells, CD8$\alpha\beta$, by coordinate binding to TCR-associated K^d molecules, substantially increases TCR ligand binding (12). Similar findings have been obtained using soluble multimeric MHC-peptide complexes and flow cytometry (13). Surprisingly, however, neither CD8$\alpha\alpha$ nor soluble CD8$\alpha\beta$ increase TCR ligand binding, even though they bind to MHC class I molecules with similar affinities (12, 14, 15). Another aspect of the role of CD8β in CD8 coreceptor function emerged from studies by Hoeveler and Malissen (4) and Irie et al. (5) showing that CD8$\alpha\beta$ associates more efficiently with lck and induces higher lck activation on cross-linking with anti-CD8 Ab, as compared with CD8$\alpha\alpha$ or CD8$\alpha\beta$ lacking the cytoplasmic tail of CD8β.

It is well established that lck plays a crucial role in T cell activation. On one hand, on activation, it phosphorylates immunoreceptor tyrosine-based activation motifs (ITAM) of CD3ζ, which permits recruitment of src homology domain 2 (SH2)-containing molecules, such as ZAP-70, Syk, and p59^{fyn} (fyn), (16, 17). lck-mediated phosphorylation of ITAM-associated kinases, e.g., ZAP-70, results in their activation (16, 17). On the other hand, lck can interact with various other signaling molecules via its SH2 and SH3 domains and thus support their recruitment to activated TCR/CD3 (18). For example, lck can bind via its SH2 domain to phosphorylated, CD3ζ-associated ZAP-70 and thus couple the coreceptor with the TCR (19). Conversely, CD8 can associate with lck, as well as with the linker of activation of T cells (LAT) (20). Thus, coordinate binding of CD8 to TCR-associated MHC molecules brings these molecules to TCR/CD3. LAT, on phosphorylation by ZAP-70, recruits a variety of adapter and signaling molecules to TCR/CD3 and hence links initial TCR activation to diverse downstream signaling cascades (21).

*Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, Epalinges, Switzerland; †Centre d'Immunologie, Institut National Scientifique et Recherche Medical-Centre National de Research Scientifique de Marseille-Luminy, Marseille, France; and ‡Basel Institute for Immunology, Basel, Switzerland

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[2] Address correspondence and reprint requests to Dr. Immanuel F. Luescher, Ludwig Institute for Cancer Research, Chemin des Boveresses 155, 1066 Epalinges, Switzerland, E-mail address: iluesche@eliot.unil.ch

[3] Abbreviations used in this paper: lck, p56^{lck}; ABA, 4-azidobenzoic acid; CD3ζ C-ITAM, third ITAM of CD3ζ; DIM, detergent-insoluble microdomain; IASA, iodo-4-azidosalicylic acid; ITAM, immunoreceptor tyrosine-based activation motif(s); LAT, linker of activation of T cells; M, membrane fraction; MCD, methyl-β-cyclodextrin; PbCS, *Plasmodium berghei* circumsporozoite; SH2, src homology 2; HIFCS, heat-inactivated FCS; PSN, penicillin-streptomycin-neomycin.

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The recognition that cell membranes are compartmentalized, i.e., contain detergent-insoluble lipid rafts, has important implications for cell activation studies. Rafts, also called detergent-insoluble microdomains (DIM), detergent-insoluble glycolipid complex (DIG), detergent-resistant membranes (DRM), or glycolipid-enriched membrane domains (GEM) are formed primarily by cholesterol and sphingolipids, which fail to integrate well in fluid phospholipid bilayers and hence form microdomains (22). Rafts are stabilized by short saturated fatty acids (e.g., palmitic and myristic acid), which intercalate in spaces between bulky raft lipids. In the outer leaflet, this is accomplished by integration of glycosylphosphatidylinositol-linked proteins (e.g., Thy-1 and CD59) (22), whereas palmitoylated and/or myristoylated proteins (e.g., lck, fyn, and LAT) play the same role in the inner leaflet (22). Other molecules, such as the abundant phosphatase CD45, however, are excluded from DIM (23, 24). Thus, by concentrating kinases and their substrates and excluding phosphatases, rafts permit efficient phosphorylation reactions to proceed and hence play a crucial role in TCR signaling. Importantly, on T cell activation with anti-CD3 Abs, TCR/CD3 translocates from phospholipid fraction to DIM (25–28). Other signaling molecules (e.g., ZAP-70, Syk, phospholipase Cγ) and adapters (e.g., Grb2 and Vav) follow this trend, at least in part due to activation-induced molecular interactions (25–28).

In the present study, we examined the roles of CD8$\alpha\beta$ and CD8$\alpha\alpha$ in TCR signaling in lipid rafts. To this end, we used T1.4 T cell hybridomas, which were obtained by fusing the T1 CTL clone with TCR-BW5147 cells (29). The T1 TCR recognizes the *Plasmodium berghei* circumsporozoite (PbCS) peptide 252–260 (SYIPSAEKI) containing photoreactive iodo-4-azidosalicylic acid (IASA) in place of PbCS S252 and photoreactive 4-azidobenzoic acid (ABA) on PbCS K259 in the context of K^d (30). Selective photoactivation of IASA permits photo-cross-linking of the peptide derivative to K^d and photoactivation of ABA to TCR (30). However, the IASA, unlike the ABA group, is not a part of the epitope and can be replaced by serine, which increases peptide binding to K^d, but not Ag recognition. We therefore refer to this peptide derivative as PbCS(ABA). Moreover, various peptide derivative variants have been tested previously on T1 and related CTL clones for Ag recognition, TCR ligand binding, and TCR-ligand complex dissociation (31). These studies showed, for example, that replacement of PbCS P255 with serine (P255S) results in a low affinity peptide variant that is inefficiently recognized by CTL, especially when CD8 is blocked (31).

Here we show that CD8 is palmitoylated at the cytoplasmic tail of CD8β and therefore, unlike CD8$\alpha\alpha$, partitions in lipid rafts. Association of CD8 with lck, which is also palmitoylated and partitions in rafts, was greatly increased in this compartment. In addition, CD8-mediated cross-linking of lck by K^d-PbCS(ABA) complexes, or anti-CD8 Ab, resulted in substantial lck activation in rafts and, in turn, in CD3ζ phosphorylation and induction of TCR signaling. Thus, palmitoylation of the cytoplasmic tail of CD8β endows CD8$\alpha\beta$ with efficient coreceptor functions.

Materials and Methods

Antibodies

Hybridomas expressing mAbs were from American Type Culture Collection (Manassas, VA). Anti-CD8α mAb 53.6.72 and 19/178, anti-CD8β mAb H35-17, anti-CD3ϵ 145.2C11, and anti-CD3ζ mAb HAM146 were purified from hybridoma supernatants by affinity chromatography on protein G-Sepharose CL-4B (Amersham Pharmacia Biotech, Piscataway, NJ). Anti-TCR Cβ mAb H57 was purified from hybridoma supernatants by affinity chromatography on protein A-Sepharose CL-4B (Amersham Pharmacia Biotech). Anti-phosphotyrosine mAb (clone 4G10) and anti-$p56^{lck}$ (clone 3A5) were from Upstate Biologicals (Lake Placid, NY). Polyclonal anti-$p56^{lck}$ was from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-LAT polyclonal antiserum was a gift from Professor Dr. C. Bron (Institute of Biochemistry, University of Lausanne, Lausanne, Switzerland).

Soluble H-2K^d-peptide complexes

Biotinylated H-2K^d/β2 m/SYIPSAEK(ABA)I complexes were expressed and purified following standard protocols (32). Multimerization was achieved by reaction with PE-labeled streptavidin (Molecular Probes, Eugene, OR) or avidin (Molecular Probes) at a 4:1 molar ratio. Tetramers were purified by gel filtration chromatography on a Superdex 200 column.

Cell culture

Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. T cell hybridomas were cultured in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 5% (v/v) heat-inactivated FCS (HIFCS, Life Technologies), penicillin-streptomycin-neomycin (PSN, Life Technologies), and 2-ME. The T1.4.1 $\alpha\beta$ and T1.4.1 $\alpha'\beta$ (α tailless) and T1.4.1 $\alpha''\beta$ (CD8α lck binding site mutant) transfectants (33) were cultured in the presence of 2 mg/ml G418 (Life Technologies). T1.4.2 $\alpha\alpha$ transfectants were cultured in the presence of 2 mM histidinol (Sigma), whereas T1.4.2 $\alpha\beta$ and T1.4.2 $\alpha\beta'$ (β tailless) transfectants were cultured in DMEM containing 1.5% (v/v) HIFCS supplemented with 2 mM histidinol and 3 μg/ml puromycin (Calbiochem, La Jolla, CA). P815 cells were maintained in DMEM supplemented with 5% (v/v) HIFCS and PSN.

Molecular biology

cDNAs coding for the wild-type CD8α gene, for the naturally occurring tailless CD8α' isoform (34), and for a mutant CD8α chain (CD8α'') in which the cysteine residues at positions 200 and 202 ($p56^{lck}$ binding site) have been mutated to alanine (4) were cloned into the expression vector pHbAPr-1 neo.

The CD8β cDNA in the LXSH vector was mutated at position 178 (TAC (Tyr)->TAG (Stop)) by site-directed mutagenesis using the QuickChange Mutagenesis Kit (Stratagene, La Jolla, CA). The open reading frame of the resulting construct encoding CD8β tailless (β') was checked by sequencing both strands. All sequencing was performed at Microsynth (Balgach, Switzerland). The construct was then subcloned into the LXSP vector and used to transfect the BOSC 23 packaging cell line (35).

Transfection and infection

Transfections of T1.4 hybridomas with protoplasts and selection in the presence of G418 were performed as described (36) to generate the T1.4.1 $\alpha\beta$, T1.4.1 $\alpha'\beta$, and T1.4.1 $\alpha''\beta$ cell lines. Alternatively, a clone that lost CD8β surface expression was obtained (T1.4.2 $\alpha\alpha$) from T1.4 hybridoma transfected with CD8α. To obtain the T1.4.2 $\alpha\beta$ and T1.4.2 $\alpha\beta'$ clones, BOSC 23 packaging cells were transfected with the CD8β or CD8β' constructs in LXSP using a standard calcium-phosphate protocol (35). After transfection, the retroviral supernatant was used to infect T1.4.2 $\alpha\alpha$ hybridomas. Puromycin-resistant populations were sorted for CD8β expression by FACS.

Flow cytometry

T cell hybridomas (5×10^5) were stained with H-2K^d/β2m/SYIPSAEK-(ABA)I/PE-labeled streptavidin tetramers in 50 μl FACS buffer (Optimem supplemented with 0.5% (w/v) BSA and 0.02% (w/v) NaN_3) for 1 h at 26°C. The cell-associated fluorescence was measured using a FACScaliber (Becton Dickinson, Mountain View, CA).

Calcium measurements

P815 cells (10^6/ml) were loaded with varying concentrations of IASA-YIPSAEK(ABA)I or of IASA-YISSAEK(ABA)I. The peptide was photo-cross-linked with K^d by UV irradiation as described (30, 31). T cell hybridomas (10^6/ml in DMEM) were incubated with 5 μM Indo-1/AM at 37°C for 45 min, washed in DMEM, and resuspended at 10^6 cells/ml. The hybridomas were then mixed with P815 cells at an E:T ratio of 1:3, sedimented by centrifugation at 1,200 $\times$ *g* for 2 min, and incubated at 37°C for 1 min. Intracellular calcium mobilization was measured on a FACStar cytofluorometer (Becton Dickinson).

Metabolic labeling

T1.4.1 $\alpha\beta$ hybridomas (10^7) were labeled with [^{3}H]palmitic acid (0.5 mCi/ml, NEN, Boston, MA) as described (37). The cells were lysed in 1 ml lysis buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% (w/v) Brij 96, 1 mM PMSF, 10 μg/ml leupeptin, 10 μM pepstatin A) for 60 min on ice. After centrifugation for 30 min at 12,000 $\times$ *g* and 4°C, the supernatants were incubated with 10 μg/ml anti-CD8α mAb 19/178, anti-CD8β

mAb H35-17, or anti-LAT antiserum and protein G-Sepharose. The immunoprecipitates were then analyzed by SDS-PAGE and fluorography.

Isolation of DIM, immunoprecipitation, and Western blotting

T cell hybridomas were lysed in 1% (w/v) cold Triton X-100 and fractionated by ultracentrifugation on discontinuous sucrose gradients, as described (37). After centrifugation, 400-μl fractions were collected from the top of the gradients and analyzed by SDS-PAGE and Western blotting. Surface biotinylation was performed as described (26). After sucrose gradient fractionation, the fractions were immunoprecipitated with 10 μg/ml anti-CD8α mAb 19/178 or anti-CD8β mAb H35-17 and protein G-Sepharose. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted with streptavidin-HRP (Life Technologies). Alternatively, T cell hybridomas (5×10^7) were lysed in 1 ml lysis buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 1% (w/v) Triton X-100, 1 mM Na_3VO_4, 50 μM (4-amidinophenyl)methanesulfonyl fluoride (Boehringer Mannheim, Mannheim, Germany), 10 μg/ml leupeptin, 10 μM pepstatin A, 2 μg/ml aprotinin) for 30 min on ice. The samples were centrifuged at 100,000 $\times$ *g* for 60 min at 4°C, and the supernatant was collected (membrane (M) fraction). The pellet was homogenized in 1 ml Brij lysis buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 1% (w/v) Brij 96 (Fluka, Buchs, Switzerland), and the same inhibitors) and incubated for 60 min on ice. After centrifugation for 20 min at 12,000 $\times$ *g* and 4°C, the supernatant was collected (DIM fraction). Membrane and DIM fractions were immunoprecipitated with anti-CD8 mAb 53.6.72 or H35-17 (20 μg) coupled to Sepharose. The samples were analyzed by SDS-PAGE and Western blotting with anti-p56^{lck} Abs.

CD8-lck association and protein kinase assays

For protein kinase assays, T cell hybridomas (1×10^7) were resuspended in 1 ml DMEM, 5% (v/v) HIFCS, and PSN and incubated with 500 ng/ml anti-CD8α mAb 53.6.72 for 10 min on ice. A secondary anti-mouse IgG Ab (10 μg/ml, Sigma, St. Louis, MO) was then added for 10 min, and the cells were subsequently incubated for 3 min at 37°C. Alternatively, the cells were incubated for 60 min at 4°C with 25 μg/ml monomeric H-2K^d-SYIPSAEK(ABA)I, UV irradiated for 90 s at 350 nm, and washed. Monoclonal antibiotin (1 μg/ml, Sigma) was added, followed by anti-IgG Ab as above, and the cells were subsequently incubated for 3 min at 37°C. The samples were then placed on ice, washed twice with 1$\times$ PBS containing 1 mM Na_3VO_4, and lysed in Brij 96 lysis buffer as above. After centrifugation, the supernatants were immunoprecipitated as indicated with protein G-Sepharose or anti-lck Ab. The immunoprecipitates were washed twice in lysis buffer and once in kinase buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 10 mM $MnCl_2$, 10 mM $MgCl_2$, 0.1% (w/v) Brij 96, 0.1 mM Na_3VO_4, 10 μg/ml leupeptin, 10 μg/ml aprotinin), resuspended in 100 μl kinase buffer containing 100 μM ATP, 10 μCi [γ-^{32}P]ATP (3000 Ci/mmol, NEN), and 10 μg/ml biotinylated CD3ζ c-ITAM peptide (38) and incubated for 5 min at 37°C. After centrifugation, the supernatant was collected, and the beads were washed twice with lysis buffer. The supernatants were pooled and immunoprecipitated with streptavidin-agarose (Pierce, Rockford, IL). After washing, the radioactivity bound to the immunoprecipitates was measured in a beta counter.

Lck and CD3ζ phosphorylation in tetramer-stimulated cells

T1.4 or T1.4.1 $\alpha\beta$ hybridomas (5×10^7) were incubated under agitation at 10^7/ml in DMEM (0.5% HIFCS, PSN, and 2-ME) with either 25 μg/ml K^d-SYIPSAEK(ABA)I tetramers or 1 μg/ml anti-CD3ϵ 145.2C11 mAb, followed by anti-IgG (10 μg/ml) for 2 h at 4°C. The cells were then washed once in medium, resuspended at 10^7/ml, and incubated at 37°C for 3 min. The samples were then placed on ice and lysed in 1% (w/v) Triton X-100, and Triton-soluble (M fraction) and -insoluble (DIM fraction) fractions were prepared essentially as described (27). M and DIM fractions were immunoprecipitated with anti-CD3ζ HAM146 (20 μg/ml) or anti-p56^{lck} polyclonal antiserum (5 μg/ml) and protein G-Sepharose. After two washes with the respective lysis buffers, the samples were analyzed by SDS-PAGE and Western blotting with anti-phosphotyrosine, anti-p56^{lck} or anti-CD3ζ Abs.

Results

T1.4 T cell hybridomas expressing CD8$\alpha\beta$ are readily activated by cognate K^d-PbCS(ABA) complexes

To investigate the role of CD8β in T cell activation, CD8$^-$ T1.4 T cell hybridomas were transfected with CD8α and CD8β genes. The resulting transfectant T1.4.1 $\alpha\beta$ expressed homodimeric CD8$\alpha\alpha$, and to a lesser degree heterodimeric CD8$\alpha\beta$ (Table I). When incubated with P815 cells, pulsed with graded concentrations of PbCS(ABA), T1.4.1 $\alpha\beta$ cells exhibited high intracellular calcium mobilization (Fig. 1*A*), which was sustained for the 10 min of recording. In the presence of anti-CD8β mAb H35-17, this calcium mobilization was reduced nearly to basal levels (Fig. 1*B*). Because T1.4.1 $\alpha\beta$ cells express high levels of CD8$\alpha\alpha$, which does not bind H35-17 mAb, this implies that CD8$\alpha\alpha$, unlike CD8$\alpha\beta$, essentially fails to promote efficient calcium flux. Pretreatment of T1.4.1 $\alpha\beta$ cells with 2-bromopalmitate, an inhibitor of

Table I. *Flow cytometry on cell lines under study*

	Transfection	Surface Marker (mean fluorescence) TCR[a]	CD8α[b]	CD8β[c]
T1 CTL		90	60	69
T1.4		97	4	4
T1.4.1 $\alpha\beta$	CD8$\alpha\beta$[d]	85	346	17
T1.4.1 $\alpha'\beta$	CD8$\alpha'\beta$[e]	52	502	12
T1.4.1 $\alpha''\beta$	CD8$\alpha''\beta$[f]	34	244	9
T1.4.2 $\alpha\alpha$	CD8α	19	104	3
T1.4.2 $\alpha\beta$	CD8$\alpha\beta$[g]	ND	129	18
T1.4.2 $\alpha\beta'$	CD8$\alpha\beta'$[e]	ND	205	18

[a] H57-259 (PE labeled).
[b] 53.6.72 (FITC labeled).
[c] H35-17 (FITC labeled).
[d] T1.4 transfected with CD8$\alpha\beta$ (from Ref. 33).
[e] Tailless (deletion of the cytoplasmic domain).
[f] $Cys_{200,202}$ of CD8α (lck binding site) mutated to alanine (from Ref. 4).
[g] T1.4.2 $\alpha\alpha$ transfected with CD8β.

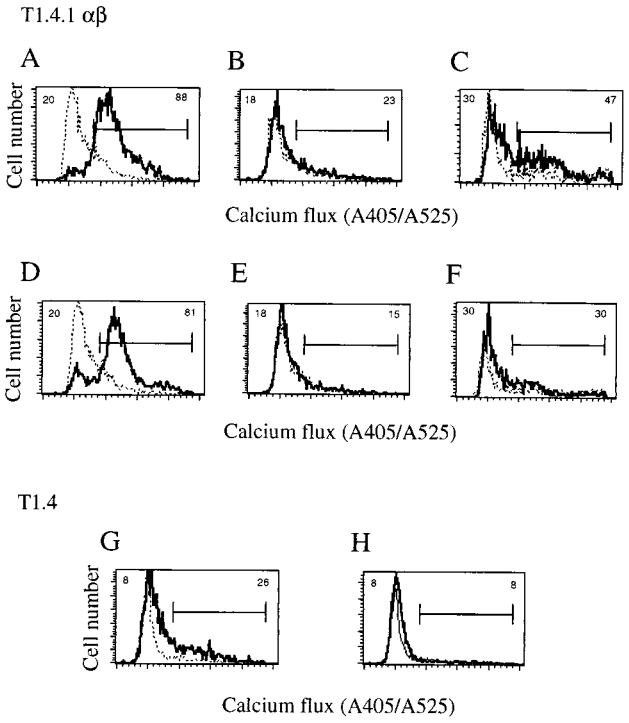

FIGURE 1. CD8$\alpha\beta$ expression endows T1.4 hybridomas to mount efficient intracellular calcium mobilization upon incubation with peptide-pulsed APC. Indo-1-labeled T1.4.1 $\alpha\beta$ (*A–F*) or T1.4 cells (*G* and *H*) cells were incubated with P815 cells that were untreated (dashed line) or pulsed with 1. 0 μM IASA-YIPSAEK(ABA)I (*A–C* and *G*) or IASA-YISSEK-(ABA)I (*D–F* and *H*) (solid line). Calcium-dependent fluorescence of Indo-1 was measured by FACS after 3 min of incubation at 37°C. In *C* and *F*, T1.4.1 $\alpha\beta$ cells were pretreated with 2-bromopalmitate (100 μM); in *B* and *E*, anti-CD8β mAb H35-17 (25 μg/ml) was present in the incubation. One of three experiments is shown.

protein palmitoylation (39), reduced the calcium flux to basal levels in most, but not all cells (Fig. 1*C*). At higher concentrations of 2-bromopalmitate, this fraction decreased, but also the cell viability (data not shown).

Similar findings were obtained for the peptide variant P255S (Fig. 1, *D–F*). In contrast, CD8$^-$ T1.4 cells exhibited only a marginal calcium flux on incubation with PbCS(ABA)-pulsed APC (Fig. 1*G*). Moreover, this calcium flux was transient, i.e., decreased gradually after 5 min of incubation. No increase in intracellular calcium concentration was observed when the peptide variant P255 was used (Fig. 1*H*). Similarly, incubation of T1.4.1 $\alpha\beta$ cells with soluble K^d-PbCS(ABA) tetramers elicited intracellular calcium mobilization (Fig. 2*A*). However, in contrast to the previous experiment (Fig. 1*A*), this calcium flux was transient and gradually decreased after 3–5 min. Again CD8$\alpha\beta$ was needed for this activation, because in the presence of mAb H35-17, or on CD8$^-$ T1.4 cells, no calcium mobilization was observed (Fig. 2*B* and data not shown). We then assessed binding of PE-labeled K^d-PbCS(ABA) tetramers to T1.4 and T1.4.1 $\alpha\beta$ cells by FACS (Fig. 2*C*). Half-maximal binding was observed at ~8 nM tetramer concentration. In the presence of anti-CD8 mAb H35-17, the staining of T1.4.1 $\alpha\beta$ cells was reduced by 20–25%, to the same levels as observed on T1.4 cells.

Taken together, these results indicate that CD8$\alpha\beta$, but not CD8$\alpha\alpha$, is required for efficient calcium mobilization in response to APC pulsed with either PbCS(ABA), the variant PbCS(ABA) P255S, or soluble K^d-PbCS(ABA) tetramers. Similar results were obtained when IL-2 production was measured. When incubated with PbCS(ABA)-pulsed P815 cells, T1.4.1 $\alpha\beta$ cells produced IL-2 more efficiently than T1.4 cells. When the variant peptide PbCS(ABA) P255S was used, only T1.4.1 $\alpha\beta$ cells displayed detectable IL-2 production, and no response was observed on T1.4 cells. Pretreatment of T1.4.1 $\alpha\beta$ cells with 2-bromopalmitate abolished the IL-2 production (data not shown).

CD8αβ, but not CD8αα, partitions in DIM

To assess the distribution of CD8$\alpha\beta$ and CD8$\alpha\alpha$ in DIM, T1.4.1 $\alpha\beta$ cells were surface biotinylated and lysed in cold Triton X-100; the lysates were fractionated on sucrose density gradients. As shown in Fig. 3*A*, CD8$\alpha\beta$ partitioned to a substantial degree in the light fractions (two and three), which contain DIM. In contrast, CD8$\alpha\alpha$ was nearly exclusively found in the dense fractions, which contain Triton-soluble components (M fractions) (Fig. 3*B*). Furthermore, lck was found in both fractions, although more in M than in DIM fractions (Fig. 3*C*). This enzyme as well as fyn can be reversibly palmitoylated on two cysteine residues near its N terminus, which affects their distribution in rafts (40–41). Because in this experiment the lysis buffer contained EDTA, which disrupts the association of CD8 with lck, the observed distributions of CD8 and lck reflect those of the nonassociated molecules. Conversely, LAT, which can be palmitoylated at two membrane-proximal cysteines (37), was found predominantly in the DIM fractions (Fig. 3*D*). In contrast, the ζ chain of the CD3 complex was found predominantly in M fractions (Fig. 3*E*). A small fraction of ζ-chain, however, was consistently observed in DIM fractions.

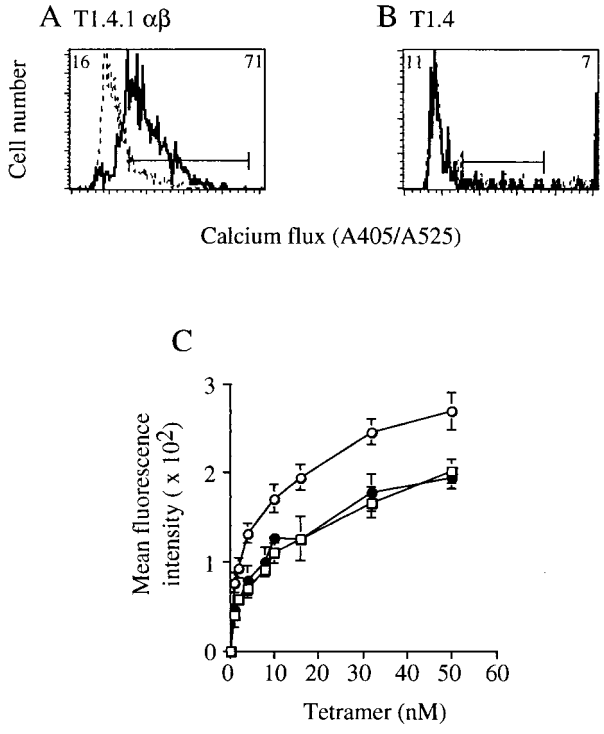

FIGURE 2. T1.4 cells expressing CD8$\alpha\beta$ exhibit intracellular calcium mobilization upon incubation with K^d-PbCS(ABA) tetramers. Indo-1-labeled T1.4.1 $\alpha\beta$ (*A*) or T1.4 cells (*B*) were incubated with K^d-PbCS(ABA) tetramers (50 nM), and calcium-dependent fluorescence was measured as described for Fig. 1. Alternatively, T1.4 (□) or T1.4.1 $\alpha\beta$ cells in the absence (○), or presence of anti-CD8β mAb H35-17 (●) were incubated for 1 h at 26°C with graded concentrations of PE-labeled K^d-SYIPSAEK-(ABA)I tetramers and analyzed by FACS. Mean values and SD were calculated from two independent experiments.

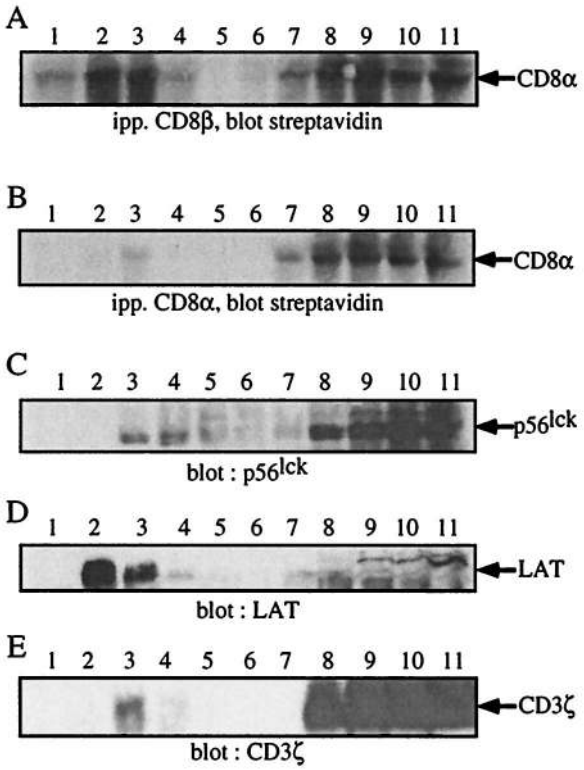

FIGURE 3. CD8$\alpha\beta$, but not CD8$\alpha\alpha$, significantly partitions into DIM. T1.4.1 $\alpha\beta$ hybridomas were surface biotinylated, lysed in cold Triton X-100, and fractionated on sucrose density gradients. The fractions were immunoprecipitated with anti-CD8β (*A*) or anti-CD8α mAb (*B*), and the immunoprecipitates (ipp.) were resolved on SDS-PAGE and Western blotted with streptavidin-HRP. Alternatively, the fractions were analyzed by SDS-PAGE and Western blotting using Abs specific for p56^{lck} (*C*), LAT (*D*), and ζ-chain (*E*).

CD8 is palmitoylated at CD8β

Because it is known that partitioning of LAT, CD4, lck, and fyn in DIM requires that they be palmitoylated (37, 40–42), we examined whether CD8 was palmitoylated. As shown in Fig. 4*A*, CD8β shares with LAT and CD4 the membrane proximal sequence CVR, which has been shown to be palmitoylated in these molecules (37, 42). CD8α also has a free membrane-proximal cysteine, but there is no consensus palmitoylation sequence with CD4 or LAT. To assess CD8 palmitoylation, T1.4.1 αβ cells were metabolically labeled with [^{3}H]palmitate and lysed in Brij 96 which solubilizes DIM at the detergent to lipid ratio used here; the detergent-soluble fraction was immunoprecipitated with Abs specific for CD8β, CD8α, or LAT. As shown in Fig. 4*B,* both CD8 immunoprecipitates displayed a major ^{3}H-labeled protein with an apparent molecular mass of ~29 kDa, corresponding to CD8β. These results indicate that CD8 is selectively palmitoylated at CD8β. The anti-LAT immunoprecipitate exhibited two palmitoylated species of 36–38 kDa corresponding to LAT (37). Only a scant amount of palmitoylated LAT was present in anti-CD8α or anti-CD8β immunoprecipitates (Fig. 4*B*), suggesting that there was no significant association of palmitoylated LAT with CD8.

Association of CD8 with p56^{lck} takes place in rafts

Because both CD8 and lck were found in DIM and M fractions, we examined in which compartment they associated. For these experiments, T1.4.2 αα cells, which do not express CD8β, were compared with T1.4.2 αβ. In T1.4.2 αβ cells, efficient association of CD8 with lck was observed only in rafts (Fig. 5*A*). In T1.4.2 αα, CD8αα-associated lck was barely detectable. Because CD8αα hardly partitions in rafts (Fig. 3), we examined whether raft localization was required for efficient CD8-lck association. This was indeed the case, because pretreatment of cells with methylcyclodextrin (MCD), which destroys rafts (25), greatly reduced association of CD8αβ with lck (Fig. 5*A*). Pretreatment of cells with 2-bromopalmitate, which inhibits protein palmitoylation (39), had the same effect (Fig. 5*B*).

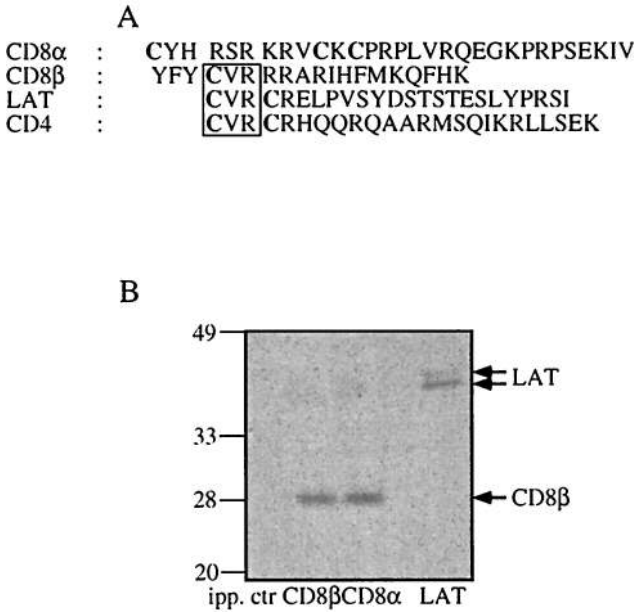

FIGURE 4. The β-chain of CD8 is selectively palmitoylated. *A*, Sequence alignment of the cytoplasmic domains of murine CD8α, CD8β, LAT, and CD4. The membrane-proximal cysteine residues are shown in bold, and the palmitoylation sequence CVR is boxed. *B*, T1.4.1 αβ hybridomas were biosynthetically labeled with [^{3}H]palmitate, lysed in Brij 96, and immunoprecipitated (ipp.) with mouse IgG (control (ctr)) or Abs specific for CD8α, CD8β, or LAT. The samples were analyzed by SDS-PAGE (10%, reducing conditions) and fluorography.

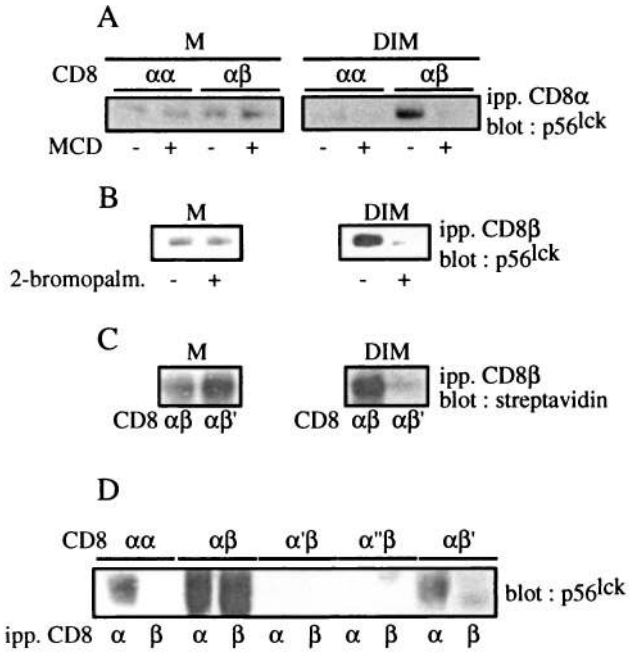

FIGURE 5. Association of CD8 with p56^{lck} takes place preferentially in rafts. *A*, T1.4.2 αα and T1.4.2 αβ cells, either untreated or preincubated with MCD (15 mM), were fractionated in M and DIM fractions, from which CD8 was immunoprecipitated with anti-CD8α mAb 53.6.72. Immunoprecipitates (ipp.) were resolved on SDS-PAGE (10% reducing) and Western blotted with anti-lck Ab. *B*, T1.4.2 αβ cells, either untreated or pretreated with 2-bromopalmitate (100 μM), were analyzed as in *A*, except that anti-CD8β mAb H35-17 was used for immunoprecipitation. *C*, T1.4.2 αβ or T1.4.2 αβ′ (β tailless) cells were surface biotinylated and fractionated in M and DIM fractions, which were immunoprecipitated with mAb H35-17. The immunoprecipitates were resolved by SDS-PAGE and Western blotted with streptavidin-HRP. *D*, Lysates of T1.4 hybridomas expressing CD8αα, CD8αβ, CD8α′β (α tailless), CD8α″β (mutation of CD8α lck binding site), or CD8αβ′ (β tailless) were immunoprecipitated with anti-CD8α or anti-CD8β mAb as indicated, and immunoprecipitates were resolved on SDS-PAGE and Western blotted with anti-lck Ab.

Moreover, deletion of the cytoplasmic tail of CD8β (CD8αβ′), which contains the palmitoylation site cysteine 179, abolished significant partitioning of CD8αβ in DIM and effective association of CD8αβ with lck (Fig. 5, *C* and *D*). As expected, association of CD8αβ with lck was abrogated on deletion of the cytoplasmic tail of CD8α (CD8α′) or mutation of its two cysteines (CD8α″), known to mediate lck binding (Fig. 5*D* and Refs. 3 and 4). Our results demonstrate that efficient association of CD8 with lck requires colocalization of both components in rafts and that the tail of CD8β increases CD8-lck association by conveying CD8 localization in rafts.

Cross-linking of CD8-associated p56^{lck} induces p56^{lck} activation

As it is established that cross-linking of CD8-associated lck results in activation of lck (5, 6), we examined anti-CD8 Ab-mediated lck activation on T1.4, T1.4.2 αα, and T1.4.2 αβ. As shown in Fig. 6*A*, cross-linking of CD8 on T1.4.2 αα cells increased the kinase activity ~2-fold above background. In contrast, on T1.4.2 αβ cells, which express about the same level of CD8αα, in addition to CD8αβ (Table I), cross-linking of CD8 with anti-CD8α mAb 53.6.72 caused a 5-fold increase in lck activity. Because mAb

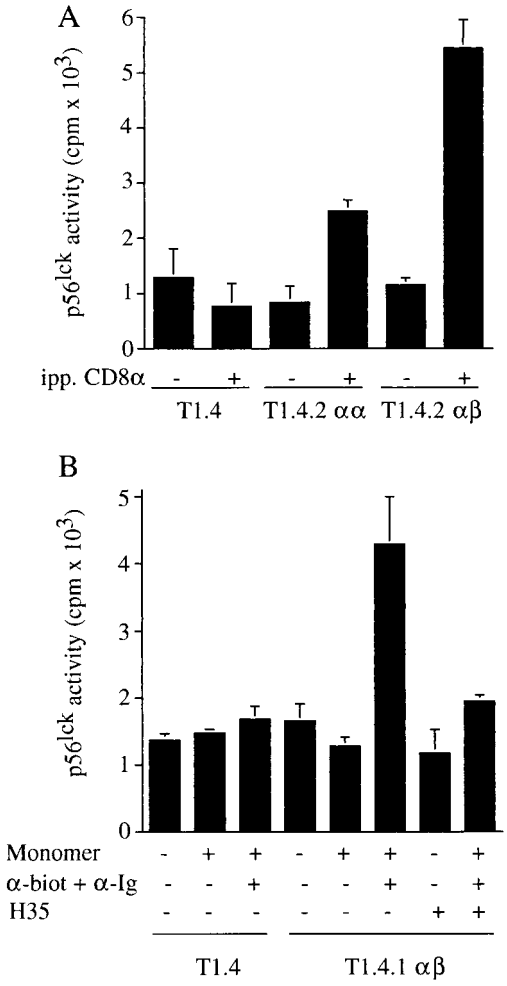

FIGURE 6. Cross-linking of CD8 by anti-CD8 Abs or H-2K^d-peptide complexes results in p56^{lck} activation. *A*, T1.4, T1.4.2 $\alpha\alpha$ or T1.4.2 $\alpha\beta$ cells were preincubated on ice with anti-CD8α mAb 53.6.72 and anti-Ig Ab, incubated for 3 min at 37°C, lysed in Brij 96, and immunoprecipitated with protein G. Kinase activity of immunoprecipitates (ipp.) was assessed in a tyrosine kinase assay using [γ-^{32}P]ATP and c-ITAM peptide as substrate. Mean and SD were calculated from three experiments. *B*, Alternatively, T1.4 and T1.4.1 $\alpha\beta$ cells, either untreated or TCR photoaffinity labeled with biotinylated soluble K^d-SYIPSAEK(*ABA*)I monomers were incubated at 37°C for 3 min with anti-biotin (α-biot.) and anti-Ig Abs. Kinase activity of immunoprecipitates with anti-lck Ab was assessed as described for *A*. Mean values and SD were calculated from two experiments.

53.6.72 cross-links CD8$\alpha\alpha$ and CD8$\alpha\beta$, this increase in lck activity was essentially accounted for by cross-linking of CD8$\alpha\beta$.

Because CD8 binds to TCR-associated K^d molecules (Fig. 2*C* and Ref. 29), activation of CD8-associated lck should also be induced by cross-linking of TCR-associated K^d-PbCS(ABA) complexes. To verify this, we photoaffinity labeled T1.4 and T1.4.1 $\alpha\beta$ cells with biotinylated soluble K^d-PbCS(ABA). As shown in Fig. 6*B*, cells that were TCR photoaffinity labeled exhibited the same low kinase activity as untreated cells. Cross-linking of TCR/CD8 by antibiotin and anti-IgG Abs increased the kinase activity substantially on T1.4.1 $\alpha\beta$, but not T1.4 cells. Antibiotin Ab alone gave only small increase in lck activity (data not shown). These findings indicate that CD8-associated lck is activated by cross-linking of CD8 by anti-CD8 mAb and, more physiologically, by multimeric K^d-PbCS(ABA) complexes.

CD8$\alpha\beta$ promotes lck activation and CD3ζ phosphorylation in T1.4.1 $\alpha\beta$ cells incubated with K^d-PbCS(ABA) tetramers

We next examined lck activation and ζ-chain phosphorylation in M and DIM fractions of T1.4.1 $\alpha\beta$ cells activated with tetramers. For simplicity, in this experiment lck activation was assessed by lck tyrosine phosphorylation, which typically increases on receptor-mediated lck activation. As shown in Fig. 7, T1.4.1 $\alpha\beta$ and T1.4 hybridomas, which are rapidly cycling cells, exhibit significant basal levels of tyrosine-phosphorylated ζ-chain (pp21) and lck phosphorylation, i.e., are constitutively activated to some degree. Incubation of T1.4.1 $\alpha\beta$ cells with tetramers resulted in translocation of lck and ζ-chain to DIM (Fig. 7*A*). This was also observed when anti-CD3 and anti-Ig were used instead of tetramers, in agreement with the results reported in other systems (25–28). After incubation with tetramer and, to a lesser extent, anti-CD3 Ab, a dramatic increase in lck tyrosine phosphorylation was observed in the DIM fraction. At the same time, the phosphorylation of ζ-chain increased, as seen by the higher amount of the pp23 phospho form. The tetramer (and anti-CD3-)-induced phosphorylation of lck and ζ-chain is in accordance with intracellular calcium mobilization observed under these conditions (Fig. 2). The pp21 and pp23 phosphoforms of the ζ-chain were observed only in DIM fractions, whereas in the M fractions the unphosphorylated p18 form prevailed.

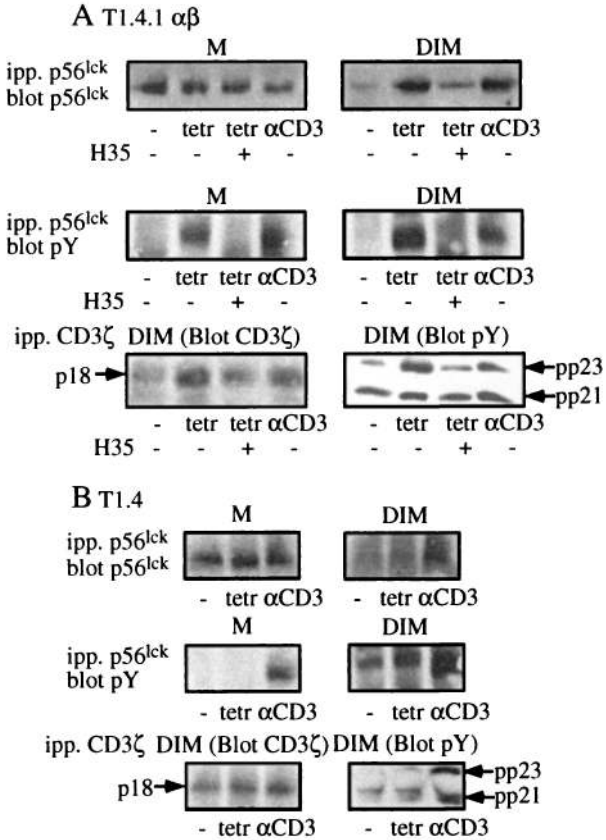

FIGURE 7. CD8$\alpha\beta$ promotes lck activation and CD3/ζ phosphorylation in T1.4 hybridomas incubated with K^d-PbCS(ABA) tetramers. T1.4.1 $\alpha\beta$ (*A*) or T1.4 (*B*) cells were incubated at 37°C for 3 min with either K^d-SYIPSAEK(ABA)I tetramers (tetr) (50 nM) in the absence or presence of anti (α)-CD8β mAb H35-17 as indicated or anti-CD3 (1 μg/ml) and anti-Ig (10 μg/ml) Ab, lysed in cold Triton X-100, and fractionated in M and DIM fractions. p56^{lck} and ζ chain were immunoprecipitated (ipp.) from each fraction, resolved on SDS-PAGE, and Western blotted with Abs specific for p56^{lck}, phosphotyrosine (pY), or CD3ζ.

In the presence of anti-CD8β mAb, H35-17 translocation and phosphorylation of lck and ζ-chain were reduced to levels slightly higher than on untreated cells (Fig. 7*A*). Thus, CD8$\alpha\alpha$, although more abundant on T1.4.1 $\alpha\beta$ cells than CD8$\alpha\beta$, was quite unable to elicit these crucial steps of T cell activation, and accordingly no calcium mobilization occurred (data not shown). On $CD8^-$ T1.4 cells, only anti-CD3 in combination with anti-IgG induced translocation and phosphorylation of lck and ζ-chain (Fig. 7*B*). Together, these results demonstrate that CD8$\alpha\beta$, but not CD8$\alpha\alpha$, promotes raft translocation and phosphorylation of lck and CD3ζ and that higher ζ-chain phosphoforms are found only in the DIM fraction.

Our data thus far indicated that CD8$\alpha\beta$ greatly promotes Ag-specific activation of T1.4 hybridomas and that palmitoylation of CD8$\alpha\beta$ is essential for this, because it mediates efficient CD8-lck association in rafts. To verify this, we examined the effect of inhibition of protein palmitoylation on K^d-PbCS(*ABA*) tetramer-induced activation of T1.4.1 $\alpha\beta$ cells. To this end, T1.4.1 $\alpha\beta$ and T1.4 cells were treated with 2-bromopalmitate and analyzed the same way as described in the previous section. As shown in Fig. 8, treatment of T1.4.1 $\alpha\beta$ cells with 2-bromopalmitate abrogated tetramer-induced translocation to DIM and tyrosine phosphorylation of lck and ζ-chain. These results are consistent with the findings that, as a result of 2-bromopalmitate treatment, CD8$\alpha\beta$ was excluded from rafts (Fig. 5) and calcium mobilization impaired (Fig. 1).

Discussion

The present study describes an important and new aspect of CD8 coreceptor function and provides a molecular explanation why CD8$\alpha\beta$ is a much more effective coreceptor than CD8$\alpha\alpha$. Because $CD8^+$ CTL express only CD8$\alpha\beta$, we took advantage of T cell hybridomas, which, when obtained by fusion of cloned CTL with the TCR^- cell line BW5147, lack CD8 expression and hence can be transfected with CD8 genes (29). Unlike CTL, CD8 transfectants of such hybridomas typically express CD8$\alpha\alpha$ homodimers and to a lesser extent CD8$\alpha\beta$ heterodimers (Table I and Ref. 12). Because CD8$\alpha\beta$ can be blocked selectively by anti-CD8β mAb, this permits comparative functional and biochemical analysis of CD8$\alpha\alpha$ and CD8$\alpha\beta$ coreceptor function.

To be able to correlate cell activation with biochemical analysis of TCR-proximal signaling events, we assessed intracellular calcium mobilization in this study, which is a much more rapid cellular response than IL-2 production. This also made possible direct comparison of T cell activation elicited by sensitized APC and soluble MHC-peptide complexes, respectively. Because activation of T cell in suspension by soluble tetramers is dependent on CD8, but not on cell polarization or auxiliary molecules, it is suitable for stringent analysis of TCR- and CD8-mediated T cell activation. Importantly, in suspension, T cells are activated by soluble multimeric, but not monomeric MHC-peptide complexes. This is true for $CD4^+$ and $CD8^+$ T cells (Fig. 6 and Refs. 13 and 43). Monomeric K^d-peptide complexes recruit CD8/lck to TCR/CD3, but for T cell activation CD8-associated lck needs to be activated first (M.-A. Doucey, D. F. Legler, N. Boucheron, J.-C. Cerottini, C. Bron, and I. F. Luescher, manuscript in preparation). It has been reported that $CD8^+$ T cells are activated by monomeric K^d-peptide complexes, but in this study the cells were adhered and not in suspension (33).

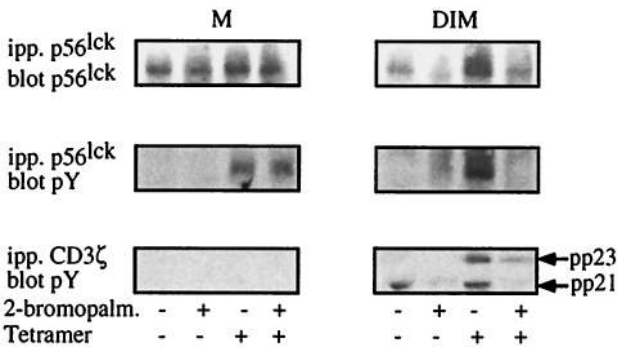

FIGURE 8. Inhibition of protein palmitoylation impairs K^d-PbCS(ABA) tetramer (tetr)-induced and CD8$\alpha\beta$-mediated activation of T1.4 cells. T1.4.1 $\alpha\beta$ cells, either untreated or pretreated with 2-bromopalmitate, were incubated at 37°C for 3 min with K^d-SYIPSAEK(ABA)I tetramers (50 nM) and analyzed as described for Fig. 7.

Our finding that CD8$\alpha\beta$, but not CD8$\alpha\alpha$, enables T1.4 cells to efficiently recognize APC pulsed with PbCS(ABA), or the weak agonist PbCS(ABA) P255S, is in accordance with numerous previous studies showing that although CD8$\alpha\beta$ is a most effective coreceptor, CD8$\alpha\alpha$ is not (1, 12). The reason for this difference is unknown, because in solution CD8$\alpha\alpha$ and CD8$\alpha\beta$ bind to MHC class I molecules with similar affinities (15). Moreover, the binding site of CD8 for lck resides in the cytoplasmic tail of CD8α (3, 4). We have previously observed that on cells, CD8$\alpha\beta$ strengthens TCR-ligand binding more than CD8$\alpha\alpha$ (12). Although these studies were performed with soluble monomeric K^d-peptide complexes (12), the CD8$\alpha\beta$-mediated increase in tetramer binding observed here is modest (Fig. 2) and seems unlikely to account for the dramatic effects observed on cell activation.

A key observation of this report is that CD8 is selectively palmitoylated at the tail of CD8β and that therefore CD8$\alpha\beta$, but not CD8$\alpha\alpha$, partitions effectively in rafts (Figs. 3 and 4). It is well established that protein palmitoylation is reversible and determines the partitioning of signaling molecules in DIM (37, 40–42). In contrast to CD8, CD4, as well as LAT, lck and fyn, have two membrane-proximal cysteines that can be palmitoylated (37, 40–42). In particular, for lck and fyn, it has been shown that the state of palmitoylation, hence their partitioning in rafts, can vary among cell types and change during cell differentiation (44). For example, the distribution of lck in rafts in T1.4.1 $\alpha\beta$ cells is considerably lower as compared with CTL or Jurkat cells (37), suggesting that lck palmitoylation is quite partial in CG72.1 hybridomas. The higher distribution of LAT in rafts, as compared with CD8$\alpha\beta$, is most likely due to dipalmitoylation of LAT, whereas CD8$\alpha\beta$ is monopalmitoylated.

For T cell activation, a crucial consequence of CD8$\alpha\beta$ and lck colocalization in rafts is the resulting highly increased association of CD8 with lck. This conclusion is based on several complementary findings: 1) CD8$\alpha\alpha$ or CD8$\alpha\beta'$, which are largely excluded from rafts, fail to effectively associate with lck (Figs. 3 and 5); 2) inhibition of protein palmitoylation by 2-bromopalmitate dramatically reduces CD8-lck association (Fig. 5); and 3) destabilization of rafts by MCD has the same effect. Because association of CD8 with lck is weak and rafts constitute only a small fraction of the cell membrane, the preferential association of the two proteins in rafts is probably mainly explained by increased concentration of both components in this compartment. Similar findings have been obtained for fyn, which associated with CD3ζ preferentially in rafts (41). We expect that the same findings apply to CD4. Because CD4 is dipalmitoylated (42), it is expected to partition in rafts more efficiently than CD8, which probably explains why CD4 associates with lck more extensively than CD8 (45).

It has been reported that CD8 (and CD4) can also associate with LAT and hence bring LAT to TCR/CD3, much the same way as lck (20). Consistent with this is the weak coimmunoprecipitation

of LAT with anti-CD8 Abs (Fig. 4*B*). According to the study by Bosselut et al. (20), however, LAT interact with the coreceptor via its two membrane-proximal cysteines, which are the palmitoylation sites. If this is correct, association of CD8 with LAT is not expected to increase in rafts, at least not in the way observed with lck.

The preferential formation of coreceptor-lck adducts in rafts has an important implication for T cell activation. Rafts, by excluding phosphatases, namely, the abundant CD45, and by concentrating kinases and their respective substrates, allow kinase-mediated phosphorylation reactions to proceed and cell activation to occur (23, 24). Because src kinases are activated by cross-linking, namely, by *trans*-phosphorylation of the regulatory A loop tyrosine 394 (24), CD8-associated lck is readily activated in rafts by cross-linking of CD8. This can be accomplished by anti-CD8 Abs and, more physiologically, by multimeric K^d-peptide complexes, which coengage TCR and CD8 (Figs. 6–8 and Ref. 13). Taking advantage of our unique experimental system, we were able to demonstrate that the initial and essential activation of lck takes place in rafts and is induced by cross-linking of TCR/CD3-CD8/lck complexes (Figs. 6 and 7; M.-A. Doucey, D. F. Legler, N. Boucheron, J.-C. Cerottini, C. Bron, and I. F. Luescher, manuscript in preparation). This conclusion is further supported by the observations that 1) monomeric K^d-PbCS(ABA) complexes failed to activate lck (Fig. 6*B*); 2) inhibition of protein palmitoylation by 2-bromopalmitate impaired CD8-lck association and lck activation in rafts (Figs. 5 and 8); and 3) CD8$\alpha\alpha$ failed to associate with lck and to mediate lck activation in rafts (Figs. 5 and 7).

As a result of this initial lck activation, tyrosine phosphorylation of CD3ζ ITAM occurs mainly, if not exclusively, in rafts (Figs. 7 and 8). In M and DIM fractions obtained from Triton X-100 lysates of resting T cells, only a small fraction of TCR/CD3 is found in the M fraction (Fig. 3 and Ref. 26). However, after activation of T cells with anti-CD3 Abs (25–28) or with MHC-peptide tetramers (Fig. 7), this fraction increases substantially, what has been referred to as translocation of TCR/CD3 to rafts (25–28). It is well established that phosphorylated ITAM are binding sites for molecules containing SH2 domains, such as ZAP-70, Syk, and fyn (16, 17). Phosphorylation of recruited ZAP-70 by lck stimulates its protein tyrosine kinase activity and promotes binding of lck to ZAP-70, thus strengthening CD8/lck association with TCR/CD3 (46). Moreover, LAT becomes firmly recruited to TCR/CD3, which is crucial because LAT, on phosphorylation by ZAP-70, recruits various adapter and signaling molecules to TCR/CD3, thus linking the initial TCR activation to diverse downstream signaling cascades (21).

Several observations indicate that these molecular interactions take place inside or at the rims of rafts. For example, the phosphatase CD45 is excluded from rafts (23, 24). In fact, due to its high abundance and high enzymatic activity, and its ability to associate with various signaling molecules, activation-induced, i.e., phosphorylation-mediated molecular interactions, can persist only when segregated from CD45. Consistent with this is our observation that activation of CD8-associated lck and phosphorylation of CD3ζ take place in rafts (Figs. 6–8). Furthermore, in a study using confocal microscopy, rafts were shown to be lined with TCR/CD3 (28). The use of cold Triton X-100 to isolate detergent-insoluble DIM is likely to disrupt weak molecular interactions that allow TCR/CD3 to interact with raft-associated molecules, such as src kinases and coreceptor. Because both lck and fyn, as well as CD8 and CD4, have been reported to weakly associate with TCR/CD3 (47), lck-coreceptor complexes, which are formed in rafts (Fig. 5), seem likely sites for TCR/CD3 association with rafts. This would explain why coengagement of TCR and CD8 by multimeric MHC-peptide complexes induces lck activation and CD3ζ phosphorylation in rafts. The subsequent activation-induced molecular interactions, resulting in the recruitment of other molecules (e.g. ZAP-70 and LAT), can be expected to further strengthen the initially weak association of TCR/CD3 with raft-associated src kinases and coreceptor, e.g., by linking coreceptor-associated lck with CD3ζ via ZAP-70 (19, 46). Thus, activation-induced translocation of TCR/CD3 and other signaling molecules to rafts (Figs. 7 and 8 and Refs. 25–28) probably reflects, at least in part, an increased stability of raft-associated molecular complexes in Triton X-100.

Palmitoylation of CD8β plays a crucial role for CD8 coreceptor function, because it allows partitioning of CD8 in rafts. This on one hand greatly favors its association with lck and on the other hand permits efficient activation of lck by cross-linking of CD8. This initial CD8-mediated lck activation is crucial for induction of TCR signaling and T cell activation, especially when Ag is limited or presented as low affinity variants.

Acknowledgments

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