The T-cell antigen receptor (TCR) exists in monomeric and nanoclustered forms independently of antigen binding. Although the clustering is involved in the regulation of T-cell sensitivity, it is unknown how the TCR nanoclusters form. We show that cholesterol is required for TCR nanoclustering in T cells and that this clustering enhances the avidity but not the affinity of the TCR-antigen interaction. Investigating the mechanism of the nanoclustering, we found that radioactively photoaffinity-labeled TCR specifically binds to the TCRβ chain in vivo. In order to reduce the complexity of cellular membranes, we used a synthetic biology approach and reconstituted the TCR in liposomes of defined lipid composition. Both cholesterol and sphingomyelin were required for the formation of TCR dimers in phosphatidylycholine-containing large unilamellar vesicles. Further, the TCR was localized in the liquid disordered phase in giant unilamellar vesicles. We propose a model in which cholesterol and sphingomyelin binding to the TCRβ chain causes TCR dimerization. The lipid-induced TCR nanoclustering enhances the avidity to antigen and thus might be involved in enhanced sensitivity of memory compared with naive T cells. Our work contributes to the understanding of the function of specific nonannular lipid-membrane protein interactions.
The mechanisms that contribute to the lateral segregation of proteins and lipids are the subject of intense research. It was hypothesized that in cells, at least two distinct membrane microdomains exist: the cholesterol- and sphingomyelin (SM)-rich lipid rafts and the phospholipid-enriched non-rafts (15). Due to post-translational lipid modifications, a number of proteins partition into the raft domain (16), where they might form clusters (17). In artificial membranes containing cholesterol, SM, and phospholipids, the formation of lateral liquid-ordered ($l_o$) and liquid-disordered ($l_d$) phases occurs (18), which might correspond to the raft and non-raft domains. This phase separation is facilitated by the interaction of cholesterol with SM (19). The actin cytoskeleton (20, 21) and protein-protein interactions (22) can also be involved in the clustering of membrane proteins.

In this report, we study the role of the lipid environment in the formation of TCR nanoclusters and the physiological relevance of TCR nanoclustering (i.e. the avidity of the TCR-antigen interaction).

**EXPERIMENTAL PROCEDURES**

**Reagents**—The following antibodies were used: rabbit anti-ζ antiserum (448), anti-CD3 (145-2C11 from J. Bluestone), anti-TCRβ (βF1, Endogen), anti-CD3ε (M20, Santa Cruz Biotechnology, Inc. (Santa Cruz, CA)), anti-transferrin receptor (TfR) (7F8, Abcam), and anti-mTCRβ (H57-598, Abcam). Secondary antibodies for Western blot and anti-mouse IgG-PE were purchased from Southern Biotech. All chemicals and reagents were purchased from Sigma if not stated otherwise.

**Generation of Expression Plasmids and Cell Lines**—To generate the expression vector pcDNA3_mζ-SBP, the DNA fragment coding for the mouse ζ chain C-terminally linked to the streptavidin-binding peptide (SBP) purification tag (23) was amplified by PCR and cloned into the EcoRI/XhoI site of the pcDNA3 vector (Invitrogen). pcDNA3_mζ-SBP was transfected into the mouse 2B4-derived ζ-deficient line MA5.8 to yield M.mζ-SBP. The cDNA of the human TIR C-terminally linked to the SBP tag was amplified by PCR and inserted into the BglII/Xhol site of the pMIG-based expression vector, pmTom (provided by R. Y. Tsien). pmTomTIR-SBP was transfected into MA5.8 cells, to yield the M.hTIR-SBP cell line. To obtain the expression vectors for the bifluorescence complementation (BiFC) assay, cDNA coding for the mouse ζ chain was C-terminally linked to enhanced GFP, the N-terminal part (residues 1–172; YN) of a yellow fluorescent protein (Venus), and the C-terminal part (residues 155–238; CC) of enhanced TCR and the NP-specific B-cell antigen receptor (BCR). Elution of the MHC molecules was covalently cross-linked to the TCR by UV irradiation. In case of the MHCp monomer, the cells were stained with streptavidin-PE. Surface TCR expression was measured by staining with FITC-labeled anti-TCRβ. Fluorescence was measured by flow cytometry with a Calibur flow cytometer (BD Biosciences). Statistical analysis was done with the Prism4 software.

**Protein Purification**—Proteins were purified from T- or B-cells expressing the appropriate construct. 5 × 10^6 cells were lysed, and affinity purifications were performed using streptavidin-conjugated agarose (GE Healthcare) in the case of SBP-linked constructs or nitrophenol (NP)-conjugated agarose (Bioseach Technologies) in the case of the sTCRβ-containing TCR and the NP-specific B-cell antigen receptor (BCR). Elution was performed by incubation for 30 min at 4 °C with 2 mm free biotin or 2 mm nitro-iodo phenol, respectively, in BNI lysis buffer containing 20 mM BisTris, pH 7.0, 500 mM ε-aminoacproic acid, 20 mM NaCl, 2 mM EDTA, 10% glycerol, and detergent as indicated.

**Thin Layer Chromatography**—A mixture of proteoliposomes (200 nmol) and 2.5% OptiPrep were added to a 3:1 mixture of CHCl3/methanol (1:1) and dried on room temperature. The pellets were resuspended in 20 μl of CHCl3 and loaded on a thin layer chromatography (TLC) plate (Silica gel 60, Merck). The TLC was run in CHCl3/methanol/NH4Cl/NH3 (9:7:2), and developed with Molybdenum Blue spray reagent.
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TCR Reconstitution in Large Unilamellar Vesicles (LUVs)—Liposomes with different membrane compositions using soybean phosphatidylcholine, egg sphingomyelin (Lipoid), and cholesterol (Sigma-Aldrich) were prepared with the thin film method (27). The lipid film was prepared by mixing phosphatidylcholine (PC), SM, and cholesterol chloroform solutions at defined molar ratios in a round-bottom flask, removing solvent using a rotary evaporator, and drying under vacuum. The film was resuspended in phosphate-buffered salt solution (3 mM Na2HPO4, 2 mM KH2PO4, 50 mM NaCl, pH 7.0) to a lipid concentration of 20 mM. Subsequent extrusion through 200-nm (21 times) and 80-nm (51 times) polycarbonate membranes (Nuclepore, Whatman) resulted in LUVs. Lipid content was determined using a phosphorus assay and Cholesterol FS assay (DiaSys). The diameters of the vesicles fell between 100 and 200 nm, as determined by dynamic light scattering (Zetamaster S, Malvern Instruments) and electron microscopy. Approximately 0.1 μg of the purified TCR in 100 μl of 0.02% Triton X-100-containing buffer was mixed with 100 μl of 2 mM LUV preparation, and 40 μg of Triton X-100 was added. Samples were agitated for 30 min at 4 °C, and the detergent was removed by adsorption to 3 mg of BioBeads SM-2 (Bio-Rad) per sample at 4 °C overnight. The same procedure was used for the generation of TfR- or BCR-containing proteoliposomes.

Flow Cytometry and BiFC—Flow cytometry for analyzing TCR expression was performed by conventional methods with a Calibur flow cytometer. For BiFC experiments, proteoliposomes were formed by separately purifying TCRs bearing the C- and the N-terminal part of the fluorophore and adding them to the performed vesicles. As controls, TCR linked to a GFP was used, and TCR linked one half fluorophore was reconstituted in itself. After TCR reconstitution, the liposomes were lysed in lysis buffer containing 1% digitonin, and an IP was performed with anti-CD3e (145–2C11) coupled to carbonate-modified latex beads (Invitrogen). Fluorescence was measured by flow cytometry and analyzed with the FowJo 8.2 software. Statistical analysis was done with the Prism4 software.

Preparation of Giant Unilamellar Vesicles (GUVs)—GUVs were made by the electroformation technique. Proteoliposomes (3 g/liter) in droplets of 2 μl were deposited on indium tin oxide-covered glass slides. The film was partially dried overnight in a desiccator under saturated vapor pressure of a saturated NaCl solution. The indium tin oxide coverslip was assembled together with a second indium tin oxide coverslip into a flow chamber of homemade design, which was filled with a buffer containing 1 mM HEPES, 1 mM NaCl, pH 7.4. Alternating electric field (400 V/m, 10 Hz) was applied for 4 h at room temperature. GUVs were observed in the same chamber. Images were collected with a Zeiss 510 Confocor3 microscope using a water C-Apochromat ×40, numerical aperture 1.2 objective and avalanche photodiodes as detectors.

Photocholesterol Labeling Experiments—[3α,5α]-3β-ol (photocholesterol) was synthesized, and the experiments were performed as described earlier (28). Jurkat cells were incubated for 16 h in lipid-free medium containing the photocholesterol-mβCD complex (5 μCi/ml) and UV-irradiated for 5 min. After cell lysis, IP and N-glycosidase F (Roche Applied Science) treatments were performed where indicated. The samples were subjected to SDS-PAGE and autoradiography.

RESULTS

Cholesterol Stabilizes TCR Nanoclusters—We established an efficient affinity purification procedure for the isolation of the native TCR from cellular lysates. We used mouse M.m/anti-SCBP cells derived from the 2B4 T cell hybridoma, expressing a SPB purification tag C-termi nally fused to the ζ chain, and human 31-13-scTCRβ cells derived from the Jurkat T cell line, which contain a single chain variable fragment of an anti-NP antibody linked to the TCRβ chain (scTCRB) (4, 24). We analyzed the effect of different detergents on the integrity of TCR nanoclusters using BN-PAGE (4, 25) in the case of both human and mouse T cells (Fig. 1A). 1% digitonin extracted the mouse TCR in monomeric form (lane 1), whereas 0.5% Brij96 extracted TCR monomers and nanoclusters (lane 2) as reported before (4, 5). In contrast, 1% saponin extracted only the nanoclusters (lane 3). The same held true for the human TCR (lanes 4–6). Brij96 extracted the remaining monomeric TCR from the saponin-insoluble membranes (lane 7). As controls, digitonin- or Brij96-extracted TCRs did not aggregate when kept in a saponin-containing buffer (Fig. 1B, lanes 1 and 2), whereas saponin- and Brij96-solubilized TCR nanoclusters broke down to monomeric TCR when digitonin was added (lanes 3 and 4). The detergent dependence of the TCR nanocluster stability suggested that membrane lipids play a role in the formation of the nanoclusters.

Previously, using harsh conditions to extract cholesterol from cells, we suggested that cholesterol might stabilize the TCR nanoclusters (4). To test whether cholesterol is involved in TCR nanoclustering, we reduced the amount of cholesterol using a short term low dose mβCD treatment (2 mM for 2 min at 37 °C), which does not extract membrane proteins (29). To increase the cholesterol content of the membranes, mβCD-complexed cholesterol was used. The change of the cholesterol content of total cell lysates was measured with the Amplex-Red cholesterol assay kit (Fig. 1C). To quantify the ratio of nanoclustered to monomeric TCRs, we lysed the cells serially in 1% saponin and 0.5% Brij96 (as in Fig. 1A). Anti-CD3ε immunopurification and SDS-PAGE were performed on the two fractions, and the amount of assembled TCR was quantified by anti-ζ WB (Fig. 1D). The nanocluster/monomer ratio decreased upon cholesterol depletion and increased upon cholesterol loading. The treatments did not extract TCRs from the membrane (Fig. 1E). These results show that TCR nanoclustering is reversible and dependent on the cholesterol content of the plasma membrane. Disrupting actin filaments by latrunculin A treatment did not disassemble TCR nanoclusters (Fig. 1F), indicating that the actin cytoskeleton is dispensable for the maintenance of the TCR nanoclusters.

The TCRβ Subunit Binds to Cholesterol in Living T Cells—Because we found that TCR nanoclusters depend on the presence of cholesterol, we used a biochemical approach to test whether the TCR can bind to cholesterol in vivo. To this end, we used a photoactivatable radioactive analog of cholesterol (28). Photocholesterol mimics cholesterol in several assays and has been used to identify proteins that specifically interact with
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FIGURE 1. Membrane cholesterol levels control the amount of TCR nanoclustering. A, different detergents extract distinct TCR forms. M.m./SBP (lanes 1–3) and 31-13.scTCRB (lanes 4–7) cells were solubilized with 1% digitonin (dig), 0.5% Brij96 (Brij), or 1% saponin (sap). In lane 7, the saponin-insoluble membranes were further lysed in 0.5% Brij96. Purified TCRs were analyzed by SDS-PAGE and anti-·IP with anti-CD3. Band intensities were quantified with the LI-COR Odyssey infrared imager, Triplicates are shown and the error bars represent the standard deviation. B, the TCR of 31-13.scTCRβ cells was purified. The washing and the elution steps were performed with a detergent different from the one used for lysis. The TCR was analyzed as in A. C, the cholesterol content of total cell lysates of the cells treated with mβCD and cholesterol was measured with the Amplex-Red cholesterol assay kit. D, the cholesterol level controls TCR nanoclustering. M.m./SBP cells were left untreated or treated with mβCD or chol. Cells were lysed in 1% saponin, and the insoluble material was subsequently extracted in 0.5% Brij96. After TCR IP with anti-CD3ε and separation on reducing SDS-PAGE, the amount of fully assembled TCR in the nanoclustered (saponin) and monomeric (Brij96) pools was determined by anti-·IP with anti-CD3ε. E, the total amount of TCR in each treatment remained unchanged (Fig. 3B, left).
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The proteoliposomes of different lipid composition were sedimented, lyzed in 1% saponin supplemented with 0.5% Brij96 to maintain or with 1% digitonin to disrupt the TCR nanoclusters, and analyzed by BN-PAGE. In liposomes containing a natural mixture of PC, the TCR remained monomeric (Fig. 5A, lane 1), whereas in PC/chol/SM liposomes (in either 80:10:10 or 40:30:30 mol % ratio) it formed dimers (lanes 2 and 3). The TCR remained monomeric in PC liposomes containing either 30 mol % cholesterol (lane 4) or 30 mol % SM (lane 5), showing that formation of the TCR dimers requires both cholesterol and SM. TCR dimers disassembled to monomers upon digitonin treatment (lanes 7 and 8). To exclude the possibility that the TCR dimers were the result of TCR aggregation after the lysis of the proteoliposomes, we mixed the TCR with LUVs but did not allow integration before lysing the vesicles and performing BN-PAGE. As expected, TCR dimers did not form without integration of the TCR into the bilayer of the PC/chol/SM-LUVs (Fig. 5B).

To assess whether dimerization in PC/chol/SM LUVs observed for the TCR is a general feature of TM proteins, we reconstituted the native purified TfR purified from the parental cell line of the M.mz-SBP cells and the purified BCR in PC or PC/chol/SM (40:30:30 mol %) LUVs. Neither TfR nor BCR multimers were detected (Fig. 5C). We also studied the effect of temperature on TCR clustering. Although we usually performed the reconstitution at 4 °C, we obtained the same results at 37 °C (Fig. 5D). In this particular experiment, the LUVs composed of PC contained more TCR than those composed of PC/chol/SM. The fact that TCR dimers formed only in the case of the ternary mixture indicated that TCR dimerization did not occur due to the congestion of a higher amount of proteins in the I1 phase.

Furthermore, we applied Bic as a detergent-independent read-out of TCR dimerization (32, 33). To the C terminus of the C chain, we fused either the N-terminal part of Venus or the C-terminal part of enhanced cyan fluorescent protein. The fusion proteins were individually expressed in M.mz-SBP cells, which already expressed SBP-tagged C. Due to C dimer for-
tion in the TCR complex, a portion of TCRs contained a half of the fluorophore after TCR purification via the SBP tag. Monomeric TCRs were purified from cellular lysates and mixed at equimolar concentrations for reconstitution into LUVs. The formation of TCR dimers in the LUVs allows the assembly of the fluorescent domain (Fig. 5E). Significantly higher BiFC fluorescence was detected from PC/chol/SM LUVs (40:30:30 mol %) as compared with PC LUVs (Fig. 5, F and G). These results confirm that the presence of cholesterol and SM in PC liposomes is sufficient for the formation of TCR dimers. As a control, we show that the tags appended to do not influence the nanoclustering of the respective TCRs (Fig. 5H).

The TCR Localizes in the Liquid-disordered Phase of GUVs—The PC and SM used in this study consisted of a spectrum of acyl chain lengths. We determined the phase behavior of the bilayers in GUVs by confocal imaging. No phase separation occurred in the case of PC or PC and cholesterol (70:30 mol %), whereas in the mixture of PC, cholesterol, and SM (40:30:30 mol %) we found an l1 phase (Fig. 6A). To determine TCR localization in the artificial membranes, we reconstituted the purified GFP-coupled TCR in PC or PC/chol/SM (40:30:30 mol %) LUVs supplemented with 0.05 mol % DiD, a dye staining the l1 domain, and grew GUVs from the proteoliposomes. Confocal imaging revealed that the distribution of the TCR was homogenous in PC liposomes, whereas in PC/chol/SM liposomes, the TCR colocalized with the l1 domain (Fig. 6B). A quantification of the distribution of the GFP-coupled TCR showed that 83 ± 11% of the TCRs was present in the l1 domain in PC/chol/SM GUVs (Fig. 6C).

DISCUSSION

The molecular mechanism of TCR nanoclustering is poorly understood. In this study, we used T cells and a synthetic biology approach to investigate the role of lipids in antigen-independent TCR dimerization. We established a procedure to purify the complete TCR complex in native form and to reconstitute it in LUVs of different lipid composition. We found that TCR dimers formed in PC/chol/SM liposomes but not in binary mixtures or in PC alone. The effect was specific to the TCR, because the TfR and BCR remained monomeric under all conditions.
Because the proteoliposomes did not contain proteins other than the TCR, we concluded that the lipid environment induced dimer formation.

A number of specific lipid-protein interactions have been revealed by x-ray crystallography (34, 35), radioactive photolipids (28, 36), and mutagenesis analyses (37, 38). Ordered cholesterol molecules were shown in the structure of metarhodopsin (39) and of the H9252/2-adrenergic G protein-coupled receptor (40, 41). Therefore, we considered that a direct interaction with cholesterol might cause TCR dimerization. Indeed, in live T cells, photoactivatable cholesterol (28) cross-linked to the TCR chain but not to any other subunits of the assembled TCR. It also did not cross-link to the BCR or to CD45. This suggests that the TCR-cholesterol interaction is remarkably site-specific and that cholesterol is a nonannular lipid-binding partner of the TCR (i.e. cholesterol might bind stably to the TCR).

Annular lipids were suggested to mediate intra- and intermolecular interactions between the TM regions involved (35, 42). In analogy, we propose a mechanism of TCR dimerization, in which cholesterol and SM serve as structural components of a TCR dimer (Fig. 6D). In most (43, 44) but not all (45) reports, the TCR was found in the non-raft phase in resting T cells. Likewise, we show that the TCR is localized in the \( l_d \) phase of the plasma membrane. Because SM preferentially interacts with cholesterol (46), cholesterol recruits SM to the TCR TM surface (Fig. 6D). The subsequent formation of TCR dimers is energetically favored, because it leads to the shielding of cholesterol-SM from the \( l_d \) phase. In addition, protein-protein interactions between TCR subunits might stabilize the TCR-TCR association (9, 47).

This model is supported by our findings that cholesterol is required for the maintenance of TCR nanoclusters. Earlier, we studied the effect of a high dose (4) and here of a mild \( \beta \)-CD treatment and cholesterol loading of T cells, and we found a correlation between cholesterol concentration in the mem-

![FIGURE 4. Integration of the TCR into LUVs.](image-url)
FIGURE 5. Nanocluster analysis of liposome-reconstituted TCR, TfR, and BCR. A, The TCR forms dimers in PC/chol/SM liposomes. M.m \( \text{m}_{\text{c}} \)-SBP cells were lysed in digitonin, and the purified TCR was reconstituted in liposomes of the lipid compositions indicated (mol %). The proteoliposomes were lysed in 1% saponin supplemented with 0.5% Brij96 or 1% digitonin and subjected to BN-PAGE and anti-\( \zeta \) WB. B, liposomes of the indicated composition were mixed with the purified TCR without integration of the TCR. The liposomes and the TCR were lysed in 1% saponin supplemented with 0.5% Brij96 or 1% digitonin and analyzed by BN-PAGE and anti-\( \zeta \) WB. An increased amount of TCR and long exposure times were used to detect the TCR. C and D, the SBP-tagged TfR (C) and NP-specific BCR (D) were purified, and the native proteins were reconstituted in liposomes of the indicated composition. After the lysis of the proteoliposomes in 1% saponin supplemented with 0.5% Brij96 or 1% digitonin, BN-PAGE and anti-TfR or anti-BCR WB were performed. D, proteoliposomes as in A were kept for 2 h at either 4 °C or at 37 °C, lysed in 1% saponin supplemented with 0.5% Brij96, and subjected to BN-PAGE and anti-\( \zeta \) WB. E, experimental procedure. Purified TCRs bearing a \( \zeta \) chain fused either to the N-terminal part of Venus or to the C-terminal part of enhanced cyan fluorescent protein were reconstituted together in liposomes. The amount of BiFC refers to the amount of dimerized TCRs in the vesicles. F, the TCR dimersizes in the presence of PC, cholesterol, and SM. Proteoliposomes containing both TCR constructs linked to the half-fluorophores (E) in a pure PC (left) or a PC/chol/SM (40:30:30 mol %) liposome (right) were lysed in 1% digitonin. The TCR was captured on anti-CD3\( \alpha \) coupled latex beads, and the fluorescence was measured by flow cytometry (green line). Proteoliposomes containing a TCR with GFP (dashed line) or a TCR with the N-terminal part of Venus only (gray) were used as positive and negative controls. G, the average of three experiments as in F is shown. A paired t test was performed (**, \( p < 0.01 \)). H, cells with TCRs bearing the different tags were lysed in 1% saponin, and the insoluble material was subsequently extracted in 0.5% Brij96. After TCR-IP with anti-CD3\( \alpha \) and separation on reducing SDS-PAGE, the ratio of the nanoclustered (saponin) and monomeric (Brij96) TCR pools was determined by anti-CD3\( \alpha \) WB. Triplicates are shown, and the quantifications are given, and the error bars represent standard deviation.
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branes and TCR nanoclustering. Furthermore, disrupting the TCR-cholesterol interaction with digitonin led to disassembly of the TCR nanoclusters.

Cholesterol amounts were measured in total cell lysates; thus, the relevant concentrations of cholesterol in the plasma membrane are unknown. However, we measured an effect of cholesterol addition or removal on the ligand-binding activity of the surface TCR (Fig. 3), demonstrating that cholesterol levels were changed in the plasma membrane.

The TCR in LUVs formed dimers but not multimers. In contrast, on the surface of T cells, larger nanoclusters are present (4, 10). Because both dimers (in LUVs) and nanoclusters (in cells) are cholesterol-dependent, we suggest that the nanoclusters derive from TCR dimers. It is unclear if the lack of nanoclusters in LUVs is due to the experimental settings (e.g. not more than two TCRs are present in one LUV) or if TCR dimers and nanoclusters form along a different mechanism.

We propose that TCR dimerization is a dynamic process in which the equilibrium between the monomers and dimers is regulated by the concentration of cholesterol and SM (Fig. 6D). The cholesterol and sphingolipid content in activated T cells is higher than in naive T cells (48). This might contribute to increased TCR nanoclustering in activated T cells as compared with naive T cells (9).

Activated T cells possess enhanced avidity, but not affinity, to MHCp tetramers when compared with naive T cells (49). Increased avidity was dependent on cholesterol (45, 49), but the underlying mechanism was unknown. Using MHCp tetramers and monomers, we show that cholesterol-mediated TCR nanoclustering translates into a higher antigen-TCR avidity. This suggests that upon activation and differentiation, naive T cells up-regulate their cholesterol and SM content, thereby expressing more nanoclustered TCRs. As a result, activated and memory T cells show enhanced avidity to MHCp (avidity maturation). In fact, activated and memory T cells posses increased sensitivity to low antigen levels as compared with naive T cells (50). Our data and the new model for TCR dimerization presented here contribute to the understanding of TM protein clustering as well as to the consequences of specific binding of lipids to TM proteins.

Acknowledgments—We thank Thomas Benzing, Bernard Schermer, and Norbert Blank for help in establishing lipid assays and Unal Coscan and Balbino Alarcon for carefully reading the manuscript. We thank Sabine Barnert and Melanie Ficht for technical help and Lipoid GmbH (Ludwigshafen, Germany) for the generous gift of lipids.

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FIGURE 6. The TCR localizes in the I LD domain of GUVs. A, confocal images of GUVs grown from different lipid mixtures of PC, cholesterol, and SM are displayed. Fluorescent Dil-C18 was used as a marker for LD domains. Confocal slices of the mixtures are shown in the first and third column. Multiple slices of the GUVs in the first column make up the projections shown in the second column. As expected, no phase separation occurred in PC or PC/chol (70:30 mol%) vesicles. In the case of PC/SM (70:30 mol%) dark finger-shaped structures were observed, representing a solid ordered domain. In PC/chol/SM (40:30:30 mol%) domains occupied half of the GUV surface, indicating the coexistence of an I LD and an I Lo phase. The diffusion of the Dil-C18 lipid marker was measured by fluorescence correlation spectroscopy. Diffusion was 3 times faster in bright I Lo domains as compared with the dark I LD domains (data not shown). B, the GFP-labeled TCR was reconstituted in LUVs composed of PC or PC/chol/SM (40:30:30 mol%). GUVs were generated from these proteoliposomes, and confocal images were taken. The LD domain was identified by DiD labeling. Scale bars, 5 μm. C, the peak fluorescence intensities of the GFP-tagged TCR in the I LD and I Lo phases were quantified using line scans through the GUVs. The percentage of partitioning (% LD = F LD/F LD + F Lo ) was calculated from 10 GUVs as described (51); the error bars represent standard deviation. D, our model of TCR dimerization. The arrangement of the transmembrane (TM) domains of the TCR subunits within the TCR complex is shown, in line with a recent publication (47). The monotonic TCR localizes in the non-raft phase (blue, left). Cholesterol specifically binds to the TCRβ subunit, which results in the recruitment of SM (middle). The presence of a cholesterol and SM islet (red) at the TM surface in the LD phase is energetically unfavorable. If the TCR forms dimers, cholesterol and SM are shielded from the LD phase, stabilizing the TCR dimer (right). The black arrows indicate the transient nature of these interactions.
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