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Role of lipid rafts in T cells

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Summary

The plasma membrane of T cells is made up of a combination of phospholipids and proteins organized as glycolipoprotein microdomains termed lipid rafts. The structural assembly of lipid rafts was investigated by various physical and biochemical assays. Depending on the differentiation status of T cells, the lipid rafts seclude various protein receptors instrumental for the early T cell signaling, cytoskeleton reorganization, protein and membrane trafficking, and the entry of infectious organisms into the cells. This review article summarizes recent information on the assembly of lipid rafts in plasma membrane of T cells and their signaling output in mature and thymic precursors towards cell growth and differentiation, and possible modalities by which the function of lipid rafts can be altered by drugs and T cell ligands. The concept of using lipid rafts as a target for pharmaceutical compounds and biological T cell ligands to ultimately alter the T cell function is discussed.

Key words: **lipid rafts • T cells • thymic selection • immune modulation**

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ASSEMBLY OF THE LIPID RAFTS

According to the fluid mosaic model of the cell membrane, the plasma membrane consists of a combination of phospholipids and proteins. To explain the lipid diversity and their interactions with proteins in plasma membrane, it was proposed that the plasma membranes of many cell types, including hematopoietic cells, contain specialized glycolipoidic microdomains. Since these microdomains are rich in sphingomyelins, glycosphingolipids, and cholesterol[12, 31, 36, 62], they have been called lipid rafts. The association of cholesterol with sphingolipids in lipid-raft microdomains promotes phase separation, apparently because of favorable packing interactions between saturated lipids and sterol[15]. The presence of cholesterol is necessary to stabilize saturated acyl chain lipids in a liquid-ordered state[13, 14]. Removal of cholesterol from plasma membranes profoundly perturbs the physical state of rafts and microdomains[35] and compromises their function[39].

Due to their high concentration of sphingolipids and cholesterol, lipid rafts exist in a liquid-ordered phase, different from the rest of the plasma membrane, which consists of phospholipids in a liquid-disordered phase. An important feature of lipid rafts in the liquid-ordered state is that their acyl chains are highly extended and tightly packed together. However, unlike the frozen gel state, in which acyl chains are even more tightly packed, liquid-ordered phase lipids exhibit high lateral mobility in the plasma membrane bilayer, since individual lipids can diffuse fairly rapidly within raft microdomains, and are even expected to diffuse between raft and non-raft microdomains.

The organization of lipid rafts restricts the access of proteins in such a way that only proteins attached to the membrane by a lipid anchor, such as glycosylphosphatidylinositol (GPI)-anchored proteins, or acylated cytosolic proteins segregate within the lipid-raft microdomains. Certain transmembrane proteins can reside in rafts, whereas the majority of integral membrane proteins are excluded[2].

Application of novel biophysical methods such as single-particle tracking to address the question of protein diffusion in membranes[64] has led to the observation that stable assemblies of outer-leaflet sphingolipids constitute confinement zones wherein GPI-anchored proteins in particular are likely to be retained[36]. The physical nature of such confinement zones was hinted at by studies showing that GPI-anchored proteins are resistant to solubilization in cold non-ionic detergents and are usually recovered as macromolecular complexes[17, 32]. These complexes are enriched in sphingolipids and cholesterol, have low buoyant density, and can be separated from the bulk of solubilized membrane lipids and proteins by gradient centrifugation[11]. The tight packing of lipids in rafts confers resistance to solubilization by non-ionic detergents at low temperatures, which allow their isolation as an insoluble membrane fraction[16]. Because of these properties and their distinct composition, lipid rafts have also been termed detergent-insoluble membranes, detergent-resistant membranes, detergent-insoluble glycolipid-rich membranes, Triton-insoluble floating fraction, or glycosphingolipid-enriched membranes[13].

Lipid rafts can be envisioned as platforms in a liquid-ordered phase that randomly move in the plane of the plasma membrane[37]. The lipid rafts represent ~40% of the immune cell membrane as estimated by fluorescence anisotropy[25]. Recently, imaging techniques have been used to visualize the lipid-raft microdomains in intact living cells, using fluorescence labeling of either proteins or lipids that are abundant in raft microdomains. Thus, rafts may be quite small in resting cells, but can be induced to coalesce into larger structures during cell activation, when the raft components are clustered through protein-protein and protein-lipid interactions[63, 70]. Estimates based on energy transfer between labeled membrane receptors suggest that in resting cells these domains are typically less than 70 nm in diameter[74] and predict a composition of not more than 10–30 proteins per microdomain[56].

The saturated acyl chains of GPI-anchored proteins are stable within clusters of liquid-ordered lipids[34, 60] and GPI-linked surface glycoproteins can be considered reliable markers for rafts.

Several pathogens utilize lipid rafts, or proteins associated with lipid rafts, for entry into cells so that they may help them evade internalization by normal means (and subsequent targeting for degradation). In particular, some viruses target lipid rafts for internalization (Simian virus 40) or as membrane fusion sites (human immunodeficiency virus – HIV)[72].

SIGNALING PROTEINS ASSOCIATED WITH THE LIPID RAFTS IN T CELLS

It has been possible to examine biochemically the protein composition of lipid rafts' specialized membrane domains. However, the degree of solubilization of lipid rafts is exquisitely sensitive not only to temperature, but also to the kind of detergent used for extraction[66].

Von Haller et al.[78] used microcapillary liquid chromatography electrospray ionization tandem mass spectrometry methodologies for the identification of proteins that co-purify with lipid rafts. Biochemical fractionation experiments have identified as well a host of molecules that are compartmentalized within the raft microdomains in T cells.

A portion of cellular Lck has a dual NH_2-terminal acylation, and is constitutively present in the lipid rafts in T cells[53, 83]. Also, LAT, a protein adaptor for T cell receptor (TCR) signaling, is targeted to the rafts by virtue of dual palmitoylation near the transmembrane region[85]. Proteins that bind to LAT, once it is phosphorylated during initiation of T cell signaling, may therefore become indirectly associated with lipid rafts[29]. Some proteins are recruited to the lipid rafts after TCR ligation, such as the phosphorylated CD3ζ chain[53]. In contrast, other proteins, such as the transmembrane tyrosine phosphatase CD45, are excluded from the rafts[57]. CD45 is a down-regulator of TCR signaling pathways[1], so its exclusion from the rafts may help control its access to the substrates, and thereby modulate the TCR signaling. These observations have led to the conclusion that lipid rafts serve not only as nucleation sites for TCR signaling modules, but also as a specific exclusion zone for TCR negative-regulatory molecules.

Some lipid rafts contain a 21–24 kDa scaffolding protein, caveolin-1. Caveolin-1 is distributed in the apical membrane of polarized MDCK cells as homooligomers, and in the basolateral membrane as heterooligomers with caveolin-2[59]. Goebel et al.[26] showed that the human macrophage-monocytic cell line U937 expresses rafts enriched in major histocompatibility complex (MHC) class II, but not class I molecules, and that this enrichment is important for tyrosine phosphorylation events within the U937 cells. Similarly, the need for clustering of class II MHC molecules in intact rafts to allow signaling into antigen-presenting cells (APCs) has been documented by Huby et al.[33] in the human myelomonocytic cell line THP-1.

Analysis of lipid-raft and non-lipid-raft microdomains prepared from unstressed Caco-2 epithelial cells indicated that Hsp70 and, to a lesser extent, calnexin, were present in the raft microdomains[10].

Flotillin-1 and flotillin-2 are also proteins constitutively associated with the lipid rafts in T cells. Slaughter et al.[65] showed increased flotillin and gangliophospholipid (GM1) expression in primary human $CD4^+$ T cells during mitogenic stimulation. When comparing peripheral blood lymphocytes from young and elderly individuals, there was a statistically significant increase in the percentage of GM1 bright-positive $CD4^+$ and $CD8^+$ T cells in elderly people. These data suggest that flotillins are important structural raft components in T cells, and changes in the GM1 expression may be a marker that reflects altered lipid-raft function in immune senescent T lymphocytes[65].

SIGNALING OF T CELLS FROM THE LIPID RAFTS

The $CD4^+CD8^+$ (double-positive) thymic precursors of T cells do not polarize lipid rafts[24]. It is thought that during the activation of T cells, the lipid-raft microdomains in the cell membrane form large macromolecular complexes[45]. Kovacs et al.[41] hypothesizes that Lck is constitutively expressed in the rafts of $CD4^+$ and $CD8^+$ T cells. Furthermore, the CD8 T cells may only require a small number of these signaling molecules at the site of activation and therefore do not aggregate lipid microdomains into large rafts. In contrast, $CD4^+$ T cells, which require participation of a larger number of TCRs for activation, might require the presence of a larger number of signaling molecules at the contact site.

Data showed that lipid rafts aggregate at the site of TCR engagement and act as foci for triggering the signaling machinery in T cells[53, 78]. In resting T cells, rafts are highly enriched in Src kinases, such as Lck and Fyn[53, 83] and LAT transmembrane adapter[85]. The CD3ζ chain is also partially associated with the rafts[53, 83]. Extensive cross linking of the TCR with antibodies promotes the rapid activation of Src kinases and subsequent accumulation in rafts of a series of newly tyrosine phosphorylated substrates[42, 47], including virtually all the hyperphosphorylated CD3ζ isoforms[43], activated ZAP-70 tyrosine kinase and phospholipase Cγ1 (PLCγ1), phosphoinositide 3-kinase (PI3-K), Vav Rac/CDC42 exchange factor[53, 83], and LAT[48, 85].

The TCR is composed of α and β chains, which bind and recognize peptide antigen presented by MHC molecules on the surface of APCs. The TCR α and β chains have a short cytoplasmic tail without kinase activity, so they cannot signal the T cells on their own, but they are noncovalently associated with the transmembrane CD3 signaling chains γ, δ, ε, and ζ. In a manner that is still unclear, MHC-peptide recognition by α and β TCR chains is sensed by the CD3 signaling chains, which then trigger the TCR signaling events. The CD3 signaling chains have no intrinsic kinase activity, but instead express a phosphorylation motif called the immunoreceptor tyrosine-based activation motif (ITAM). Upon TCR engagement by

antigen-loaded MHC molecules, ITAMs on the CD3 chains become phosphorylated by the Src-family kinase Lck and become docking sites for the cytoplasmic Syk-family kinase ZAP-70. Once recruited to the phosphorylated ITAMs, ZAP-70 becomes phosphorylated and activated, and in its turn is responsible for phosphorylating many proteins associated with the T cell signaling pathways. Some of the pathways activated downstream of ZAP-70 include PLCγ1[73], PI3-K[80], the adaptor protein SLP-76[81], and LAT[85]. Lin et al.[48] proved that LAT must be present in the lipid rafts to activate the Ras pathway, to increase the intracellular Ca^{2+}, and to stimulate the NF-AT-dependent transcriptional activity in response to TCR engagement. The extracellular and transmembrane domains of LAT are not required for these signaling modules once LAT is localized into the rafts. The CD28 ligation can enrich the rafts in LAT, while cytotoxic T lymphocyte antigen 4 (CTLA-4) ligation blocks LAT-mediated signaling from the rafts[51]. Phosphorylated LAT serves as a docking site for PI3-K, PLCγ1, and Grb2[85, 86], and is critical for coordination of T cell activation response. Together, these pathways ultimately induce transcription of genes which promote cell cycle entry, including the T cell growth factor, interleukin 2 (IL-2)[37].

The negative regulator of Lck, known as Cbp or PAG, is also located in the rafts, where it functions to recruit cytosolic Csk, which is a negative regulator of Src-family kinases to the membrane[8]. The bulk of Lck present in the rafts of resting T cells is inactive because of Csk kinase that is localized in the rafts by binding to Cbp, another raft-resident protein[9, 38]. Csk can negatively regulate the Lck kinase by phosphorylation, thereby causing Lck to enter an inactive conformation[5]. The phosphatase activity of CD45 is actually required to remove this inhibitory COOH-terminal phosphate from Lck and render it active. Presumably, the Lck fraction present in lipid rafts is protected from CD45 phosphatase activity and is inactive as a result of CD45-mediateed dephosphorylation[57].

Both the CD4 and CD8 co-receptors were found in raft microdomains that contain GPI-anchored proteins, such as CD59 and CD48. Many protein kinase C (PKC) isoforms have been shown to translocate from the cytosol to membrane rafts upon PMA treatment[55]. PKC, an isoform that is selectively expressed in T cells[4] and plays an important role in T cell activation, can translocate to lipid rafts in T cells after anti-CD3/CD28 stimulation[6]. When expressed in T cells, the Nef protein of HIV-1 was also found to associate with lipid rafts and with an increase in IL-2 production upon stimulation with anti-CD3 plus CD28 antibodies. Both of these activities of Nef required its myristoylation[79].

The co-receptors CD28 and CTLA-4 have opposing effects on TCR/CD3 activation of T cells[51].

The findings by Coudronniere et al.[19] that PKCθ integrates TCR/CD28 costimulatory signals leading to NF-κB and CD28 RE activation, together with data showing that CD28 enhances T cell activation by promoting lipid-raft clustering[77], led to the examination of the relationship between PKCθ and lipid rafts in the context of the T cell synapse[52]. It was found that upon T cell activation by anti-CD3/CD28 antibodies, PKCθ translocates to the lipid rafts. Also, in intact T cells, PKCθ translocates to membrane patches induced by antibody-mediated cross-linking of GM1 with cholera toxin. Similarly, engagement of antigen-specific, TCR-transgenic T cells with peptide-bound APCs led to co-localization of PKCθ and membrane rafts at the contact area (the immunological synapse). The translocation of PKCθ to lipid rafts required Lck, but not ZAP-70, and the raft-resident PKCθ was tyrosine-phosphorylated and physically associated with Lck. An agent that disrupts lipid rafts abolished the CD3/CD28-induced, Lck-mediated tyrosine phosphorylation of PKCθ, and also inhibited the activation of NF-κB[6]. These results indicate that physiological T cell activation translocates PKCθ to membrane rafts, which localize to the T cell synapse in antigen-stimulated T cells and, moreover, that this translocation is important for the proper function of PKCθ.

ROLE OF LIPID RAFTS IN THE THYMIC SELECTION OF T CELLS

A functional immune system requires elimination (negative selection) of self reactive T cells, and propagation of T cells responding to foreign antigens (positive selection). This selection occurs predominantly in the thymus. Progenitors of T cells are provided by the bone marrow as $CD4^{-}CD8^{-}$ double-negative cells that enter the thymus near the cortical-medullary junction[49]. As the cells migrate toward the subcapsular epithelium, they upregulate CD25 and downregulate CD44. At this point, TCR rearrangement occurs, and pairing with pre-TCR results in downregulation of CD25 and proliferation with a simultaneous expression of CD4 and CD8 co-receptors ($CD4^{+}CD8^{+}$, double-positive cells)[68]. The DP cells are the first population to express TCR heterodimers on the cell surface. The positive and negative selection occurs in the medullary thymus, from where they migrate to the periphery as $CD4^{+}CD8^{-}$ and $CD4^{-}CD8^{+}$ single-positive T cells.

Positive and negative selection allows cells with a functional TCR to leave the thymus. Selecting peptides with low-affinity binding for TCR are presented to thymocytes by thymic epithelial cells and APCs, and promote the positive selection. Stronger-affinity peptides for TCR are thought to promote negative selection by apoptosis-induced cell death, leading to clonal deletion. The negative selection also occurs by passive apoptosis induced by a lack of growth factors (cell death by neglect)[3, 18, 44, 61]. Passive apoptosis cannot distinguish between self reactive and naïve T cell precursors. However, it seems that the rules of negative selection do not apply to a particular subset of T cells, namely the regulatory T cells which, in contrast to other T cell subsets, require a strong TCR stimulation to be positively selected.

Generation of antigen-specific repertoire in T cells devoid of self reactivity assumes that TCR would transduce qualitatively different signals at different stages of thymic development. Some data suggest that the association of protein receptors with lipid rafts may change during development and differentiation, presumably to regulate the outcome of signaling. Thus, a large amount of immature pre-TCR in $CD4^{-}CD8^{-}$ double-negative thymocytes was found to reside in lipid rafts, unlike the mature T cells, where the TCR is excluded from lipid rafts[28, 58]. Indeed, in immature T cells the surrogate chain of TCR is palmitoylated[58], which provides the biochemical basis for association with lipid rafts. In contrast, during the negative selection, the TCR is excluded from the rafts in resting thymocytes, and is not recruited in lipid rafts following TCR ligation[24, 46].

Thus it appears that the lipid rafts play an important regulatory role on the thymic development of T cells. Little is known about the assembly and composition of proteome in the lipid rafts of T cell precursors, particularly in self reactive T cell precursors. At present, there is a single report showing that the lipid rafts do not localize in the plasma membrane of thymocytes as they do localize in mature T cells[24]. We found distinct patterns of GM1-raft distribution in the T cell precursors at various stages of thymic differentiation and ages.

Lipid rafts and the T cell cytoskeleton

Villalba et al.[76] showed that Vav/Rac-dependent cytoskeleton reorganization is required for lipid-raft clustering in T cells. The proper function and locomotion of lymphocytes require intact function of the actin cytoskeleton. It is well established that T cell activation induces actin polymerization[20, 22] and that agents which disrupt the actin cytoskeleton inhibit T cell activation[71]. The actin cytoskeleton regulates the shape as well as the movement of antigen-specific T cells, and it is essential for formation of the T cell synapse[23]. In addition, the cytoskeleton acts as a scaffold for the recruitment of different signaling molecules to the T cell synapse. Accordingly, peptide/MHC stimulation of T cells reorients the actin cytoskeleton towards the contact site with APCs[82].

A number of studies suggests that the lipid rafts and the actin cytoskeleton are functionally, if not physically, linked. It has been shown that the lipid rafts localize to the contact area between T cells and anti-CD3/CD28-coated beads[49] or to the T cell-APC contact area in antigen-stimulated T cells[7]. Furthermore, polymerized actin is enriched in lipid-raft patches induced by cross-linking of GM1 glycosphingolipid with its specific ligand cholera toxin B in T cells[30]. This process depends on the Lck tyrosine kinase, and intracellular tyrosine-phosphorylation of proteins also accumulated in these patches[30]. The lipid rafts seem to be the place where TCRs become associated with actin cytoskeleton, since disruption of lipid rafts by methyl-β-cyclodextrin (MCD) was found to abolish association of ζ chain with the actin cytoskeleton[54].

Beyond the co-localization of polymerized actin and lipid rafts in activated T cells, little is known about the functional relationship between these two cellular compartments. In particular, it is not clear whether reorganization of the actin cytoskeleton is required for optimal lipid-raft clustering or, conversely, whether lipid-raft clustering plays a role in promoting actin cytoskeleton rearrangements. Some studies support a role for lipid rafts in actin cytoskeleton reorganization. The finding that treatment of T cells with PP1, a selective inhibitor of Src-family kinases (which are required for actin polymerization in T cells), does not prevent CTx-induced patch formation[30] suggests that lipid-raft clustering can occur independently of actin cytoskeleton reorganization. This notion is also supported by the finding that T cells treated with latrunculin, an inhibitor of actin polymerization, still form lipid rafts upon CTx-mediated GM1 cross-linking, albeit these patches are less condensed than in untreated cells. These results support a model in which initial clustering of lipid rafts is independent of actin polymerization and PTK activation, although accumulation of F-actin under the rafts may stabilize the rafts. However, the major caveat of this model is that in these studies, clustering of lipid rafts was induced by artificial means, i.e. passive, CTx-mediated cross-linking of GM1-containing membrane microdomains.

In contrast to CD28 costimulation, passive aggregation of lipid rafts by cross-linking of raft-associated molecules (GM1, CD59) does not provide a strong costimulatory signal for T cells, since it lacks the TCR/CD3 stimulatory signals[77]. Nevertheless, the notion that the lipid rafts are required for cytoskeleton-associated signaling events in TCR/CD3-activated T cells is supported by the finding that disruption of lipid microdomains by MCD treatment inhibits tyrosine phosphorylation of CD3ζ chain and prevents its association with the actin fibers[54].

Some evidence supports the notion that the actin cytoskeleton plays a role in recruiting and stabilizing the lipid rafts[23]. In this case, lipid-modified signaling molecules (e.g. Src-family kinases), which are associated with the cytoskeleton, may be co-localized to rafts and thus may function as "handles" to mediate cytoskeleton-driven rearrangement of the rafts[84].

DISRUPTING THE ARCHITECTURE OF LIPID RAFTS IN T CELLS

The functional importance of lipid rafts in T cell signaling has also been demonstrated by disruption of lipid rafts, using several agents. The most widely used one is MCD, an agent that extracts cholesterol from the membrane. Treatment of Jurkat T cells with MCD resulted in reduction of Ca^{2+} mobilization and tyrosine phosphorylation induced by TCR stimulation[83]. Depletion of cholesterol by MCD also locks natural killer cell activation[50].

Filipin and nystatin, polyene antibiotics that specifically associate with cholesterol, have also been used to disperse lipid rafts and were found to inhibit TCR activation including calcium response and tyrosine phosphorylation of CD3 and PLC-1 induced by OKT3 stimulation[83]. Nystatin was previously shown to block internalization of GPI-anchored surface protein CD59[21] through disruption of lipid rafts. Both MCD and filipin can disperse the clustering of IL-2 receptor and its co-localization with HLA and CD48 on T lymphoma cells[75].

As mentioned previously, the plasma membrane lipid rafts are rich in sphingolipids with saturated acyl chains, causing rafts to reside in a liquid-ordered environment. Polyunsaturated fatty acids (PUFAs) were found to function as immunosuppressive agents. The inhibition of T cell activation by PUFAs strictly correlates with their ability to remove Src-family kinases from lipid rafts, suggesting that the immunosuppressive effects of PUFAs may be due to their ability to alter the composition of the inner leaflet of rafts and disrupting their localization by S-acylation-mediated incorporation into the protein receptors[40, 69].

Several immunosuppressive drugs widely used in humans are now believed to act through a raft-mediated mechanism, e.g. glucocorticoids diffusing across membrane and binding to intracellular receptors that alter nuclear gene transcription[40]. Also, statins, which are cholesterol-reducing agents, can destabilize the rafts in plasma membrane[27].

We found that ligation of T cells with various antibodies specific to TCR and CD4 co-receptor, or a combination of these antibodies, induced reorganization of the proteome in lipid rafts of resting T cells

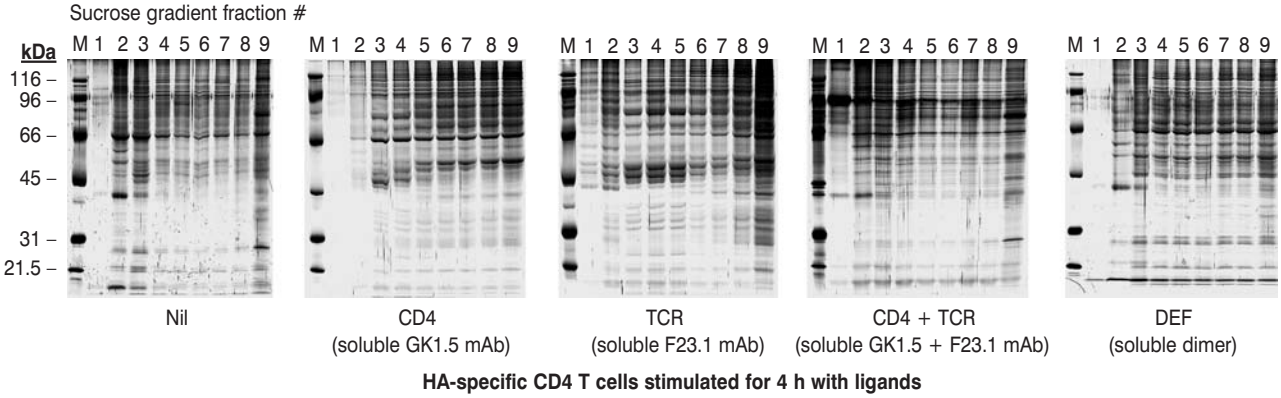

Figure 1. Proteomics of lipid rafts from T cells cross linked with various ligands. Negatively sorted CD4 T cells from transgenic mice expressing a T cell receptor (TCR) specific for HA110-120 peptide of PR8 virus were stimulated for 4 h at 37°C with 5 μg/ml/10^6 cells of soluble CD4 mAb (#GK1.5, ATCC), TCR Vβ8.1 (#F23.1 mAb, ATCC), an equimolar combination of CD4 and TCR Vβ8.1 mAbs, or a soluble dimeric HA110--120 peptide-MHC II (I-E^d) chimera. Cells were washed in PBS to remove the ligands, then lysed, and the cell membranes were separated by centrifugation for 2 h at 120,000 x g. The membrane pellet was solubilized in Brij 58-based buffer containing a cocktail of protein inhibitors and centrifuged for 18 h at 4°C in a sucrose gradient as described[70]. Nine fractions were collected from the top of the tube and analyzed by SDS-PAGE in 8–16% gradient polyacrylamide under denaturing and reducing conditions. The protein pattern was revealed in individual fractions by silver stain.

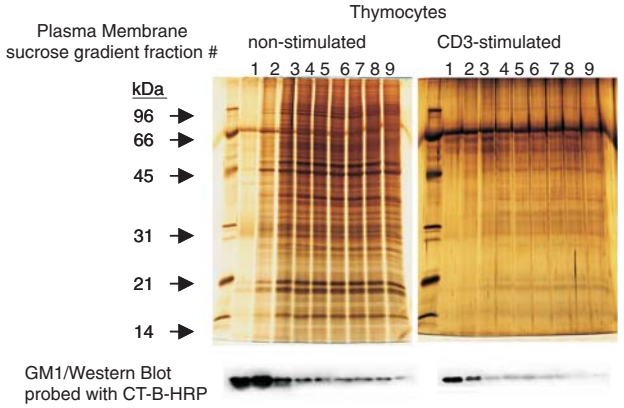

Figure 2. Patterns of protein distribution in plasma membrane microdomains of thymocytes stimulated with immobilized CD3 mAb. Total thymocytes from 3-week-old BALB/c mice were stimulated (left panel) or not (right panel) for 2 h with plated-2C11 mAb (10 μg/ml/10^6 cells), washed, lysed, and the cell membranes were separated by centrifugation for 2 h at 120,000 x g. The membrane pellet was solubilized in Brij 58-based buffer containing a cocktail of protein inhibitors and centrifuged for 18 h at 4°C in a sucrose gradient as described[70]. Nine fractions were collected from the top of the tube and analyzed by SDS-PAGE in 8–16% gradient polyacrylamide under denaturing and reducing conditions (upper panel), and by Western blot using CT-B-HRP conjugate (lower panel).

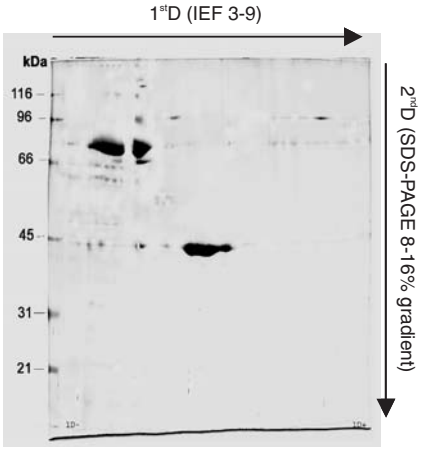

Figure 3. 2D map of proteins from GM1-rich rafts isolated from thymocytes. The GM1-positive sucrose gradient fractions from plasma membrane of thymocytes (fr # 1–3) were pooled and separated by isoelectric focusing (pI 3–9) in the 1st dimension, and in SDS-PAGE 8–16% gradient gel under reducing and denaturing conditions in the 2nd dimension.

(Fig. 1). Furthermore, we showed that the nature of receptor cross-linking is a parameter that dictates the assembly of protein receptors within the raft microdomains. Thus, a simultaneous cross-linking of TCR and CD4 on T cells by a soluble, dimeric peptide-MHC II chimera (DEF) induced unique alterations of the proteome in the lipid rafts that were not obtained with a combination of TCR and CD4 antibodies. DEF dimer induced a displacement of CD4--p56lck signalosome from the lipid rafts of T cells leading to anergy of T cells, which could not be

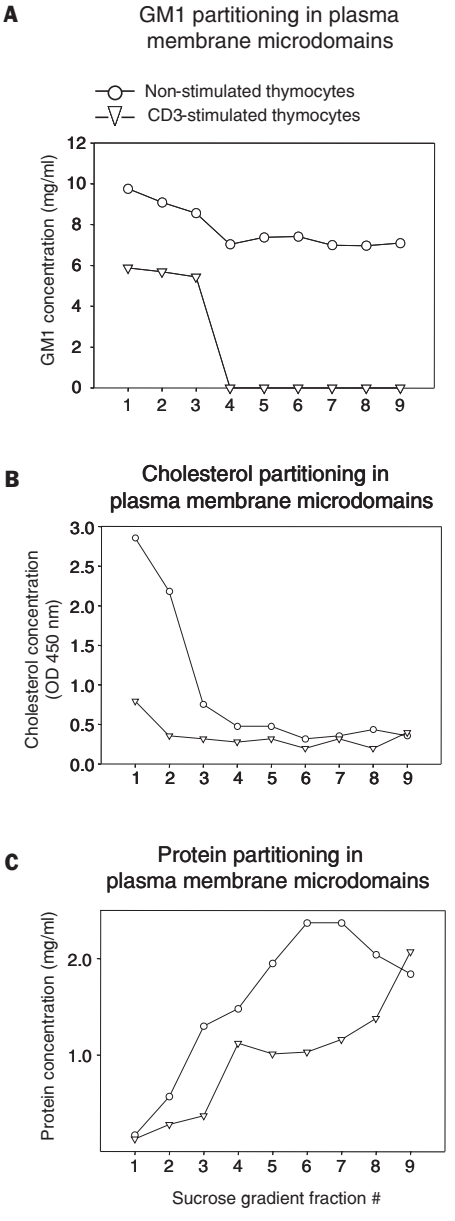

Figure 4. Alteration of GM1, cholesterol, and protein partitioning in plasma membrane lipid rafts from thymocytes stimulated with CD3 mAb. Thymocytes were stimulated or not for 2 h with plated-2C11 mAb (10 μg/ml/10^6 cells), washed, lysed, and the cell membranes were separated by centrifugation for 2 h at 120,000 × g. The membrane pellet was solubilized in Brij 58-based buffer containing a cocktail of protein inhibitors and centrifuged for 18 h at 4°C in a sucrose gradient as described[70]. Nine fractions were collected from the top of the tube, the sucrose was dialyzed out, fractions were individually concentrated to 200 μl, and measured for GM1 content by a sandwich ELISA using 96-well plates coated with CT-B and revealed by CT-B-HRP conjugate (**A**), cholesterol content by ELISA (**B**), and for the protein content by Bradford microassay (**C**).

induced by a combination of TCR Vβ8 and CD4 antibodies[70].

Ligation of CD147 induces a displacement of GPI--anchored co-receptors CD48 and CD59 from

microdomains in human T lymphocytes[67]. This perturbation of microdomains is accompanied by a selective inhibition of TCR-mediated T cell proliferation. The CD147-inhibited cells secrete normal levels of IL-2 but express reduced amounts of IL-2 receptor α chain (CD25). These results indicated that various protein receptors can promote negative regulatory signals in T cells throughout the lipid-raft microdomains in plasma membrane of T cells.

Using sucrose gradient centrifugation fractions from plasma membrane of thymic precursors and mature T cells from BALB/c mice, we found that the proteomics of lipid rafts are quiet different in thymocytes and in mature T cells (Fig. 2). A 2D map of GM1 positive plasma membrane fraction (pooled fractions 1–3) using isoelectric focusing for the 1st dimension and SDS-PAGE for the 2nd dimension showed a different profile of the proteins (as detected by silver stain) in the rafts from thymocytes and mature T cells (Fig. 3). We also found that CD3 ligation of thymocytes induced a decrease in the content of GM1 glycosphingolipid, cholesterol, and proteins in plasma membrane. Also, the distribution of proteins in plasma membrane microdomains was quiet different after CD3 stimulation (Fig. 4). Partitioning of lipid rafts and the proteome of lipid rafts can thus be greatly affected not only by cholesterol-disrupting drugs, but also by various T cell ligands.

In conclusion, the lipid rafts are well conserved structures in the plasma membrane of various cells. They appear to follow rules of assembly and segregation of protein receptors that are currently under investigation. Understanding how these structures influence the biology of T cells may provide rational grounds for the future development of a new class of drugs and biological reagents aimed at modulating the T cell function in pathological conditions such as infectious diseases, cancer, and autoimmune diseases.

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