Review

The Functional Roles of Lipid Rafts in T Cell Activation, Immune Diseases and HIV Infection and Prevention

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The first appearance of lipid rafts, or lipid rafts-like structure, was occasionally observed by cryo-electronic microscopy in 1980s as cavity, such as caveolae. However, the fully understanding of lipid raft was attributed by the studies of T cell activation, virus entry/budding, and other membrane events. During the interaction of T cell and antigen presenting cell, a highly organized structure is formed at the interface of the two cells, where cholesterol and sphingolipids are enriched, and form a liquid ordered phase that facilitates the signaling proteins on and off. Lipid rafts are also involved in virus entry and assembly. In this review, we will discuss cholesterol-sphingolipid floating microdomain, the lipid raft as a unique compartment of the plasma membrane, with biological functions that ensure correct intracellular traffic of proteins and lipids, such as protein-protein interactions by concentrating certain proteins in these microdomains, while excluding others. We also discuss the disruption of lipid rafts is related to different diseases and aging, and we especially exploit the lipid rafts as pharmaceutical targets for anti-virus and anti-inflammation, particularly a new approach to control HIV infection for AIDS prevention and protection by inhibition or disruption of lipid rafts. *Cellular & Molecular Immunology*. 2008;5(1):1-7.

Key Words: lipid raft, liquid ordered phase, liquid crystal phase, cholesterol, virus assembly

Introduction

For a long period the fluid mosaic model proposed by Singer and Nicolson in 1972 was regarded as the dogma structure of plasma membrane (1). The concept of lipid raft was introduced by Brown and Rose in 1992 (2), and rafts were visually observed by Simon and Ikonen in 1997 (3). Lipid rafts are widely regarded as dynamic assemblies of selected proteins, cholesterol and sphingolipids that exist in the exoplasmic leaflet of cellular bilayer membranes. As sphingolipid and cholesterol have less tendency of liquidation, a structural environment that fits for the conformation change of signal transducers, immune-receptors and other proteins, is created. One of the most distinguishable biophysical characteristics of lipid rafts from other plasma membrane is their resistance to mild detergents. They can be easily isolated by equilibrium centrifugation in sucrose gradient, or visualized by image or video with immuno-fluorescent staining and microcopies. The ligation of T cell antigen receptor (TCR) with MHC II molecules of antigen present cell (APC), a general mechanism for activation of immune cell signaling in T lymphocytes, occurs in lipid rafts, which forms a supramolecular activation cluster (SMAC), or an immunological synapse (Figure 1A), a dynamic structure during the T cell activation (4). In addition, many viral pathogen infections take place at the site of lipid rafts. For example, human immunodeficiency virus (HIV), SV40, influenza virus and other viruses use lipid rafts as the injection sites of nucleotide acids, and the sites for assembly and budding as well.

Structure of lipid rafts

The fluid mosaic constructure of plasma membrane is regarded as disordered fluid liquid crystal (L_c) phase membranes with phospholipids enriched and is a semipermeable lipid bilayer, an excellent external barrier of cell membrane, while liquid ordered (L_o) rigid phase is densely organized with cholesterol and sphingolipids in outer membrane immersed in the phospholipid environment. The lipids and proteins of the lipid raft are assembled in the trans

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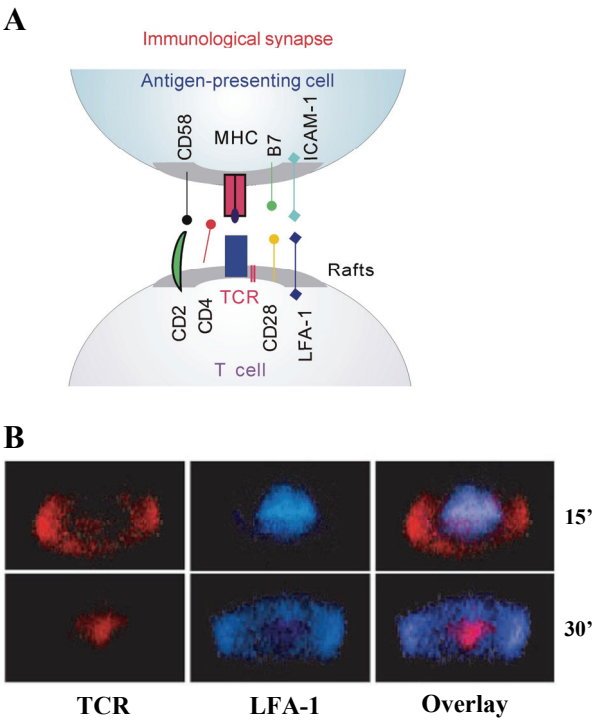

Figure 1. The central event of T cell activation occurs in lipid rafts of the conjugation of T cell and APC. (A) A diagram that discribes the main components involved in TCR mediated T cell activation, which occurs in the lipid rafts of plasma membrane. (B) *z*-stack reconstruction of the T cell - APC interface at 15 and 30 min after conjugate formation. At 15 min, the TCR (red) is localized in a peripheral ring, whereas LFA-1 (blue) is in the center (immature immunological synapse). At 30 min, the TCR is now in the center, surrounded by LFA-1 in an outer ring (mature immunological synapse, see reference 7).

Golgi network (TGN). Cholesterol depletion or inhibition of glycosphingolipid synthesis blocks the formation of secretory vesicles from the TGN. Interestingly, it has been demonstrated by ESI/MS that arachidonic acid (AA), a substrate of prostaglandin synthesae (PTGS), or cyclo-oxygenase (COX), is also enriched in the lipid raft segment (5). AA is frequently loaded or unloaded according to the demand of muscle motion, gastric lining, inflammatory, anti-inflammatory stimulation and other activities. The cholesterol depleting reagent methyl-β-cyclodextrin (MBCD) was shown to suppress signal transduction that was triggered by endocannabinoid analogue R (+)-methanandamide (R-MA), which suggests that R-MA induces cyclooxygenase (COX)-2 expression in human neuroglioma cells *via* a pathway linked to lipid raft microdomains because MBCD is a strictly surface-acting reagent and selectively extracts plasma membrane cholesterol (6). Another characteristic of lipid raft is its dynamics, for instance the TCR molecules are located inside the SMAC 15 minutes after conjugation begins, while the adhesion molecule, lymphocyte function-associated antigen-1 (LFA-1) is present outside the rafts, but LFA-1 proteins turn inside, TCR turns outside the SMAC after 30 minutes (Figure 1B) (7). The dynamic is due to the unique structure and function of lipid rafts, which present a special electropermealization and electroinsertion suitable for conformation change of the proteins (8). As matter of a fact, TCR does not constitutively reside in membrane lipid rafts, only moving into the rafts when physiologically T cell activation truly occurs *in vivo*.

Lipid raft proteins can be purified from the pellet of cell extract solublized with 1% Triton X-100, or 0.45% Nonidet P-40, or 0.45% Tween 20 at 4°C and centrifuged at 10,000× g. The lipid raft fraction can be collected and analyzed by suspension, immune precipitation, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and western blotting with intended antibodies (9). Since possible artifacts of lipid rafts can be present in such a detergent resistance membrane (DRM) method, so an alternative approach is to colocalize a protein with a raft marker by microscopic examination (confocal microscopy, fluorescence resonance energy transfer microscopy, electron microscopy, etc.). However, in most cases, rafts can be only visualized in nanometer, or in micrometer after clustering with raft protein components. Disruption of the membrane rafts by treatment of the cells with a cholesterol chelator MBCD or a cholesterol sequestering agent will lead to loss of the association of raft proteins. For *ex vivo* observation, the lipid rafts can be visualized by green fluorescence protein (GFP) that has been engineered to link a palmitylated peptide, thus palmitylated GFP can be inserted into lipid rafts for real time live video record (10). Typically the proteins in lipid rafts can move in any direction or turn inside or outside. The structure of cholesterol in lipid rafts is able to interact with some protein motif, mainly to the phenolic structure of unfolded peptide, such as specifically recognizes HIV gp41 protein amino acid LWYIK motif (11).

Lipid rafts facilitate TCR mediated T cell activation

The activation of resting T cells is crucial to most immune processes. Recognition of foreign antigen by T cell receptors has to be correctly translated into signal transduction events, a necessary step for effective immune responses. T cell activation is a central event in the protective adaptive immunity and in the maintenance of self-tolerance. During T cell activation, the antigen is processed and presented by APC to T lymphocytes through MHC classes I/II molecules. The formation of the TCRαβ-MHCαβ complexes and aggregation of costimulatory ligands in lipid rafts (Figure 1A) are engaged on the surface at the T cell-APC junction, and subsequently induce dramatic T cell polarization by dephos-phorylation/phosphorylation and the formation of a specialized immunological synapse. The conjugation can hold together up to several hours. In the engagement of a cascade of downstream signaling events, raft aggregation promotes tyrosine phosphorylation and recruitment of signaling proteins, but excludes certain proteins, such as the tyrosine phosphatase CD45 and CD43. TCR-MHC ligation gives

strong evidence that lipid rafts are important in controlling appropriate protein interactions in resting and activated T cells, and that aggregation of rafts following receptor ligation is a structural dependent/associated mechanism for promoting immune cell signaling. The resultant effector T cell population comprises a greatly expanded set of antigen-specific T cells. Most effector cells die after the pathogen is cleared, while a small fraction emerges and persists as memory T cells (12). Correct T cell activation is a must for normal physiological condition, otherwise autoimmune or other diseases may occur.

The kinase's phosphorylation or autophosphorylation of tyrosine, serine, or threonine leading to conformation change for CD3 and the accessory proteins is largely restricted to lipid rafts. One of the most dynamic molecules is CD45 that plays an important role in T cell activation. CD45 as a receptor phosphatase is a controversy non-raft protein because CD45 is abundant in T cells, but normally not detected by the lipid raft analysis methods. However, by far, CD45 is necessary to trigger the SMAC and signaling as one of the most effective factors in initiating reconstituting T cell activation, and then it rapidly leaves the rafts after its tyrosine dephosphorylation for lck and others. The dynamic movement of lipid rafts can be also seen from the pair-up of the CD28 and CD80/CD86 (or B7.1/B7.2), CD4 and many accessory molecules during $CD3^+$ T cell activation (13). However, in T cell activation and other lipid rafts involved events, ganglioside GM1 is more commonly used as rafts marker, which can be detected by fluorescence labeled cholera toxin B subunit (CTB), a B-pentamer that can be bound to GM1 ganglioside receptor, even though GM1's role in T cell activation is probably a structural protein (14). Today fluorescence-CTB, anti-GM1 antibody, or CTB-green fluorescent protein (CTB-GFP) is commercially available for detection of lipid rafts in cell biology. In addition, lipid rafts can be divided into buoyant cholesterol and cholesterol-rich rafts. Non cholesterol-rich lipid rafts in CD3/TCR signaling can optimally transmit CD3/TCR signals as well because there is evidence of full CD3/TCR T cell activation after mild cholesterol-depletion with MBCD (15). Similar to TCR of T cell, MHC I and II of APC have been also reported to be raft proteins (16).

Lipid rafts in inflammatory diseases

Lipid rafts play important physiological roles in T cell activation and signaling transduction for immune defense. Lipid rafts are also involved in many inflammatory diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. The clear evidence is that proinflammatory or inflammatory factors, such as the receptors of lipopoly-sacchride (LPS). CD14, Toll-like receptor and interleukin receptors are concentrated in lipid raft fraction in the cells treated with LPS. Lipid raft integrity has been seen essential for LPS-cellular activation, since raft-disrupting drugs, such as nystatin or MCD, inhibit LPS-induced tumor necrosis factor α secretion (17). The lipid raft involved in LPS induced inflammation indicates that disruption of lipid rafts can be an anti-inflammatory approach in pharmacology rather than currently implemented targeting cyclooxygenase (COX)-1, or 2 or 3 (18), which has shown different side effects with either selective or non-selective inhibition (19).

Lipid rafts are related to aging, typically to the β-amyloid peptide associated Alzheimer. One of the evidences is that cholesterol content of the T cell plasma membrane was significantly increased in the aging tissues in normo-lipidaemic individuals (20). The clear difference between naïve and memory cells and/or effector cells in terms of lipid raft distribution and protein content has also been observed. Naïve T cells have fewer rafts in their plasma membrane and require CD28-B7 costimulation to amplify TCR signaling. By contrast, effector and/or memory T cells have more rafts in their plasma membrane, amplification of signaling is thus able to occur in the absence of CD28-B7 costimulation. As matter of fact the aging does not affect too much of the population of each group of lymphocytes, but evidently on their functions, particularly in TCR mediated signaling, which is influenced by the distribution of different amount of lipid rafts in the membrane, eventually causing different immune diseases.

Lipid rafts in HIV infection and prevention

Lipid rafts as platforms for virus HIV-1 entry

HIV-1 entry takes place in lipid rafts because the binding of HIV-1 envelope gp120 to lipid rafts only happens in the presence, not in the absence, of cholesterol (21). The HIV-1 envelope protein is synthesized from a precursor gp160. During its transport to the cell surface, the fraction of gp160 is cleaved into gp120 and gp41. The external gp120 is responsible for the attachment to the cellular receptors and coreceptors, whereas the transmembrane protein gp41 is responsible for the fusion of viral envelope with the plasma membrane of the target $CD4^+$ T cells. HIV-1 gp120 attaches to a CD4 receptor of T cell, or macrophage, and exposes the gp120 V3 loop to further interaction with the chemokine coreceptor CCR5 and/or CXCR4, triggering conformational changes of gp120, and gp41 that ultimately lead to the fusion of the viral and host cell membranes (Figure 2). CCR5 is a lipid raft protein because it can be easily co-immuno-precipitated with CD4, whereas CXCR4 is not unless cells have been preincubated with soluble gp120. During adsorption at 37°C, HIV-1 initially binds to CD4 in a raft domain, secondary association with CXCR4 requires the shift of proteins and associated lipids away from their preferred lipid environment. The membrane fusion of gp41 induces a structural protein flotillin 1 enriched in lipid rafts, which leads to destabilization of the plasma membrane, but may favor the fusion reaction. Rafts facilitate HIV-1 adsorption onto CD4 and then disperse prior to the ultimate membrane fusion reaction or would stimulate transient CXCR4 motion into rafts as a result of CD4 signaling (22).

Lipid rafts probably contain certain cellular automata interaction/recognition machinery. The highly conserved LWYIK (679-683 of gp160) motif is located immediately

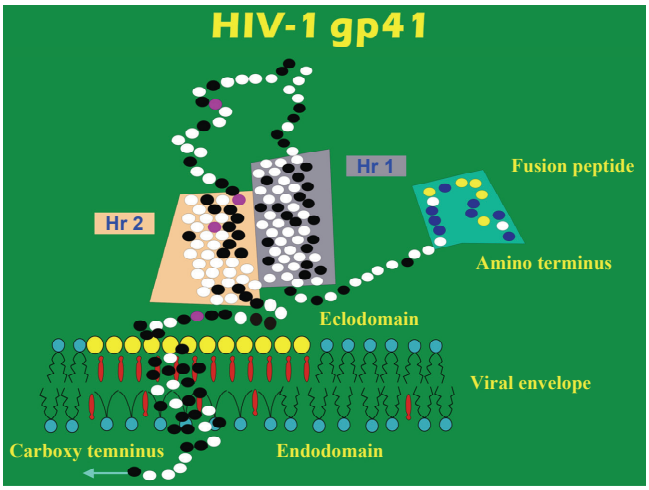

Figure 2. gp41 conformation in the lipid rafts of the plasma membrane during HIV-1 entry. A diagram that presents cholesterol (red bar) and sphingolipid (yellow cycle) enriched in outer membrane as basic structure for lipid rafts that fits protein signaling and viral entry, assembly and budding. The diagram also postulated a HIV-1 entry mechanism in which gp41 is involved in conformational changes. The LWYIK motif (679-683) recognition with cholesterol in lipid rafts assists the insertion of transmembrane peptide (685-709). Highly conserved gp41 heptad repeat regions (Hr-1 and Hr-2) topological conformational change leads to HIV-1 and target T cell membrane fusion induced by fusion peptide. All the events happen in lipid rafts (modified from Dr. O'Brien 2001 with his permission).

proximal to the membrane spanning domain of the HIV-1 gp41, which allows LWYIK motif to specifically recognize the cholesterol in rafts, and ensures the fusion and other events in HIV-1 entry and assembly (23). Some proteins in lipid rafts are anchored to the membrane by a lipid moiety associated either by palmitylation or actylation, for example, glycosylphosphatidylinositol (GPI)-anchored proteins are palmitoylated into the extracellular leaflet, while lck, a doubly acetylated protein, is enriched in the inner cytoplasmic leaflet. However, geranylated proteins are excluded from rafts. With artificial membrane the external subunit gp120 of HIV failed to bind to the cholesteryl-resin, but soluble form of the HIV-1 envelope glycoprotein gp160 devoid of the transmembrane anchor domain was found to bind to cholesteryl-hemisuccinate agarose, further suggesting that the binding site with cholesterol was located in the gp41 transmembrane protein (685-709 of gp160). It is worthy to point out that HIV-1 nonproductively infects brain microvascular endothelial cells *via* a macropinocytosis mechanism that is dependent on lipid raft integrity and on the mitogen-activated protein kinase signaling pathway (24).

Lipid rafts as platforms for virus HIV-1 assembly

Nef has been defined as HIV assembly factor. Nef is targeted to membrane rafts by its myristoylation and positive charge at its N-terminus. This allows Nef to be present in virus particles and enriched together with GM-1, a chimeric Nef-N-terminus-green fluorescent protein is efficiently incorporated into HIV particles. Interestingly, *in vitro*, Nef is able to bind to RNA and to reverse transcriptase (25). The targeting of Nef to membrane rafts can favor these interactions, where HIV-1 assembly occurs. Taken together, rafts represent a necessary step during HIV-1 assembly. With similar way of both assembly and budding within membrane rafts many other viruses including influenza virus, measles virus, Ebola virus, and possibly Sendai virus also use lipid rafts as assembly platforms. There are a few characterized processed proteins for viral assembly the protein Env-gp41 is located in lipid raft fraction when HIV-infected cells were extracted with cold Triton X-100. Polyprotein precursor $Pr55^{gag}$ is also associated with membrane rafts, and can be easily colocalized with GM1 (26). In the presence of normal amount of cholesterol, Nef significantly enhances HIV-1 infectivity, and this effect is abolished when virus is produced from cholesterol-depleted cells. Likewise, cholesterol depletion or sequestration from HIV-1 particles inhibits virus internalization, probably by preventing the fusion step. In contrast to the cholesterol depletion of target cells, incubation of cholesterol-depleted HIV-1 with cholesterol did not result in a recovery of virus internalization (27).

Lipid rafts as platforms for virus budding

After viral assembly in the TGN, the naked nucleocapsid viruses are packed by host cell plasma membrane. Enveloped viruses contain a host cell-derived lipid bilayer acquired during budding. Virus detachment from cell is described as a pinching-off step. This requires local curvature of the membrane to form a bud, followed by forming a neck (or lipid stalk) and fission. Although the lipid rafts have ability to regulate budding out of vesicles from the TGN, the mechanisms by which the lipid raft can favor the budding and/or fission process is a matter of speculation. Membrane fusion is energetically unfavorable and, it is almost certain that the stalk contains a protein machinery to mediate the fission step. Besides the structure of lipid rafts energy is also needed. HIV-1 release is blocked and virus buds are anchored by a stalk accumulation in the presence of ATP-depleting agents (28). Treatment of human T lymphocytes cultured in cholesterol-poor medium with lovastatin, an inhibitor of cholesterol synthesis, inhibits HIV-1 production (29). Lovastatin is a cholesterol lowering agent isolated from a strain of *Aspergillus terreus*. After oral ingestion, lovastatin, as an inactive lactone, is hydrolyzed to the corresponding beta-hydroxyacid form. This is a principal metabolite and an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate limiting step in the biosynthesis of cholesterol. The exclusion of the abundant nonraft CD45 phosphatase from the HIV-1 envelope and the incorporation of raft lipid components ganglioside GM1 and resident proteins (the GPI-anchored proteins Thy-1 and CD59) indicate that HIV-1 specifically buds from rafts (30). The blocking of HIV-1 budding after treatment of cells with unsaturated fatty acid directly indicates a critical role of rafts in virus budding. This

treatment inhibits $Pr55^{gag}$ targeting to rafts without affecting its association with cell membranes and significantly reduces the number of virus-like particles released into the supernatant (31). The Nef protein, which is recruited into the virus at the raft assembly site, enhances virus release and infectivity (32). Thus, Nef probably participates directly in the formation of the budding scaffold as well.

HIV is able to incorporate large numbers of cell-derived proteins when budding from the location of lipid rafts. More than 20 different host cell-derived proteins have been identified in the HIV-1 envelope, including major MHC-I and MHC-II, the adhesion molecules CD44, LFA-1, 2, 3, and ICAM-1, 3. These virion-associated, host cell-derived proteins can serve as markers to identify the type of cells from which a virion budded. The molecular phenotype of the HIV virion envelope has been used to determine whether HIV virions produced *in vivo* budded from a macrophage, or an activated T cell. Incorporation of host cell-derived proteins into virions doesn't seem to be random, nor simply due to differential expression level or density on the cell surface, since proteins highly expressed on HIV infected cells, such as CD4, CD45, or even other lipid raft coreceptors CXCR4, CCR3, and CCR5, are not incorporated into virions, which are implications of viral pathogenesis and immunoregulation of budded viruses (33).

The inhibitors of lipid rafts for antivirus

At present most HIV inhibitors target reverse transcriptase, or protease, for instance tenofovir is a nucleotide reverse transcriptase inhibitor and saquinavir is HIV protease inhibitor. They are small molecules that inactivate the enzymes. Since fusion inhibitor T-20 (enfuvirtide 36 AA), a surrogate/analog to heptad repeat 2 (HR2) in the gp41 helical region that prevents HR1-HR2 interaction and correct foldings, was approved by FDA in March 2003, there has been increasing interests in the identification of new HIV entry inhibitors that may target various stages of the HIV entry/fusion process with less side effects because this approach is to keep virus outside the door (34, 35). T-20 binds the first HR subunit of the viral envelope protein and prevents conformational changes required for membrane fusion. HR1 appears to become accessible to T-20 after Env binds CD4, whereas coreceptor binding is thought to induce the final conformational changes that lead to membrane fusion, thus T-20 binds to a structural intermediate of the fusion process. Primary viruses exhibit considerable variability in T-20 sensitivity. The affinity of gp120 and the coreceptor is correlated with T-20 and coreceptor antagonist sensitivity, with greater affinity resulting in increased resistance to both classes of entry inhibitors. Enhanced affinity results in more rapid fusion kinetics, reducing the time for the necessary interaction of T-20 and HR1 (36, 37).

There are other types of lipid raft inhibitors that deplete cholesterol, such as MBCD and lovastatin. Since cholesterol is necessary for HIV entry and assembly, depletion of cholesterol, such as MBCD has been shown the anti-HIV activity in a dose dependent manner as a cholesterol-lowering agent. A structurally similar compound called simvastatin can also decrease HIV replication/production. The cholesterol reducing drugs, the statin class, already in widespread clinical use, has been shown to modulate T cell responses, affecting immune T cell activation by influencing membrane cholesterol levels necessary for the function of lipid rafts. Lovastatin also inhibits replication of HIV by inhibiting the interaction of ICAM-1 on virions with LFA-1 on the lipid rafts of target cells. At present it is still a question if it is possible to screen some naturally derived small molecules that directly disrupts the lipid rafts. However, each step of the virus entry procedure could be a potential target for novel antiviral agents termed virus entry inhibitors, even possibly inhibition between the interaction of LWYIK of gp41 and cholesterol in lipid rafts.

For blocking HIV-1 entry, protein disulfide isomerase (PDI) has been becoming another important target because PDI is closely located beside CD4 receptor in HIV-1. This could enable PDI to reduce gp120 disulfide bonds during the virus entry of $CD4^+$ T cell. The reduced structure-stabilizing disulfide bonds (-SH + SH-) leading to conformation changes increase the gp120 interaction with coreceptor and with the small conserved loop of gp41. This will affect the interaction of HR1-HR2 within gp41, which may lead to failure of virus infection. Several small molecules targeting PDI have been entered phase II clinic trial. If the clinic trials are successful this will largely reduce the cost of chemotherapy of AIDS patients comparing to the current fusion inhibition of T-20 class of peptide drugs (38).

Summary

Lipid rafts are highly dynamic entities: within minutes of receptor or ligand stimulation, phosphorylation or dephosphorylation that cause raft protein composition and conformation drastically changes, with only a small percentage of proteins remaining invariable, which have been proved to be excellent life machinery. Recently in HIV DNA vaccine design the major HIV-1 raft proteins, such as Nef or Gag are also used as antigenic epitopes (39). All in all lipid rafts have being used as pharmaceutical target to develop new drugs for anti-immune disorder and antivirus with the strategies on inhibition of virus entrance and exit. Even though more precise structure and function of lipid rafts need to be further explored, it has provided tremendous clues to use lipid raft inhibition as useful experimental tools or to screen rafts inhibiting therapeutic agents for HIV prevention and protection.

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