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Float on: lipid rafts in the lifecycle of HIV

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1. ABSTRACT

All steps in the HIV lifecycle – entry, assembly, budding, induction of signal transduction and subsequent cell activation – are complex multifactorial mechanisms where cholesterol and sphingolipids (glycosphingolipids – GSLs and sphingomyelins in mammalian cells) are closely involved. Here we will review the contribution of these heterogeneous membrane lipid microdomains, referred to as lipid rafts, DIGs (detergent-insoluble glycolipid-enriched complexes), DRMs (detergent-resistent membranes), GEMs (glycolipid-enriched membranes), caveolae, TIMs (Triton-insoluble membranes) for interactions of HIV with the host cell. The accurate terminology was discussed elsewhere (1), and to simplify matters we will use rafts or lipid rafts throughout the review.

2. INTRODUCTION

Lipid rafts support many lipid-protein and protein-protein interactions and are central for the transport of materials in, through and out of cells. Rafts also play an important role in the immune response. Despite their role in immune cell activation, these host microdomains are abused to target host cells not only by retroviruses (e.g. HIV, MoMLV, HTLV), but also by measles virus, influenza virus and other viruses, bacteria and protozoa. The microdomains of the host cell are exploited by the pathogens at different stages during infection. They are encroached as entry and exit portals, as replication sanctuaries and efficient assembly areas within the cells, and as basis to manipulate signaling pathways in order to evade immune responses. This review will provide an

overview on the organisation and signaling capacity of lipid rafts, summarize the interactions of rafts and HIV at the cell membrane level (entry, assembly, exit) and in the last part deal with HIV-induced modulation of cellular signal transduction via lipid rafts. In the last few years a number of reviews discussed lipid rafts, lipid raft-pathogen interactions, and immunological or virological synapse formation (1-11). Thus, we will mainly focus on recently published papers to give an overview of lipid rafts and on so far known manipulation mechanisms of signal transduction in the host cell by HIV, as the overwhelming majority of publications is dealing with this human retrovirus.

3. RAFTS – OVERVIEW

3.1. Composition and function of lipid rafts

In recent years evidence accumulated indicating that membrane rafts are constitutive for the lateral organisation of the lipid bilayer that is composed of phospholipids, cholesterol and sphingolipids. The voids between the sphingolipids (GSLs, sphingomyelin) are filled up by cholesterol molecules thus resulting in a dynamic cluster in the cell membrane bilayer, referred to as lipid rafts (12). Saturated molecules of sphingolipids and cholesterol associate laterally to a liquid-ordered phase (l_o phase), in contrast to the liquid cristalline or liquid disordered phase (l_c or l_d phase) of the fluid lipid bilayer (13). Rafts are mainly present in apical plasma membrane domains, and in lower concentrations also in the basolateral plasma membrane and on Golgi and *trans*-Golgi membranes.

Membrane fractions containing clusters of many lipid rafts can be isolated by treatment with TritonX-100 or other mild detergents at low temperatures (4°C), whereby they form detergent-insoluble glycolipid-enriched complexes (thus called DIGs or DRMs) that float to a low density during gradient centrifugation due to their high lipid content (14). Recently questions were raised whether these microdomains are relevant for living cell membranes or whether they are displaying an artefact generated during extraction at low temperatures with TritonX-100. Due to these concerns a number of advanced techniques were performed to detect membrane microdomains *in vivo* and to characterise the nature of lateral organisation of specific lipids and proteins at the cell surface and in artificial membrane systems. Damjanovich *et al.* (15) and Lagerholm *et al.* (16) reviewed different sophisticated techniques (e.g. FRET, electron microscopy in combination with immunogold-techniques, confocal laser scanning microscopy, scanning force microscopic techniques, single-particle tracking, microdot nanotechnology) to show that lipid rafts are not only an *in vitro* artefact but are also present in living cells.

Due to the small size of single rafts and their limited capacity to carry only a determined set of proteins, laterally diffusing rafts coalesce to form platforms, where mediation of intracellular sorting processes (e.g. in the *trans*-Golgi network or endocytic pathway), signal transduction or immunological synapse (IS) formation take place (17-19).

Rafts play an essential role in signal transduction and cell activation (20-22). Specific proteins, e.g. positive signal transducers such as Src-family protein tyrosine-kinases (LAT, Fyn, Lck), preferentially localise in lipid rafts, whereas others, such as negative regulators of signal transduction like CD43 and CD45, are specifically excluded. Other proteins associated with lipid rafts are GPI (glycosylphosphatidylinositol)-anchored proteins (e.g. CD59, CD55/DAF, CD14, CD16, CD90/Thy-1), transmembrane proteins (e.g. LAT), MHC class I and MHC class II molecules, chemokine and other cell surface receptors (e.g. CXCR4, CCR5, TCR, BCR, FcRs), caveolin [only in caveolar rafts], and annexin (23-27). Raft association of proteins can be assayed by cholesterol depletion. Previously raft-associated proteins dissociate after treatment with the cholesterol-depleting reagent β-cyclodextrin and become detergent-soluble (28). Drugs inhibiting sphingolipid synthesis showed that in addition to cholesterol, sphingolipids are essential components for formation of lipid rafts. Lipid depletion has been shown to disturb sorting and signaling properties of many membrane proteins.

Proteins associated with rafts are linked via two ways, either as GPI-anchored proteins or through acylation with myristate or palmitate (17).

Upon stimulation, the small dynamic rafts cluster into larger platforms with sustained signal transduction. Aggregation of GPI-anchored proteins, either initiated by binding of ligands or antibody-mediated cross-linking, can recruit new proteins to raft regions to create a signaling centre (17). Cross-linking of GPI-anchored proteins on the extracellular raft-moieties shifts Src-family protein tyrosine-kinases at the inner leaflet, initiating autophosphorylation of the kinases and thus activation of signaling cascades (29).

3.2. Signaling capacity of rafts

The small size of lipid rafts in resting cells might be essential to keep the signaling molecules switched off, while upon cross-linking rafts coalesce; then they are often attached to the cytoskeleton and may become microscopically detectable (30-32). These clustered rafts are composed of adapters and additional signaling components and play a pivotal role in signal transduction. Association of clustered rafts with the actin cytoskeleton mediates stabilisation of the complexed molecule and involvement of rafts in immune receptor signaling has been extensively studied for TCR, BCR, and FcεRI (33-35). TCR events take place in a narrow cleft between T cell and antigen-presenting cell (APC), which is referred to as immunological synapse (IS) (rev. in 36). When TCRs recognize their specific MHC/peptide complex on the APC and aggregation occurs, they become associated with raft microdomains containing protein tyrosine kinases (PTKs), which can phosphorylate and activate the immuno-receptor tyrosine-based activation motifs (ITAMs) (29). Under resting conditions, the TCR-CD3ζ–complex is excluded

from lipid raft platforms. Ligation of the TCR translocates the complex into the microdomains, where accumulation of PTKs as well as CD3ζ-chains results in initiation of signaling cascades (37). After formation of the initial T cell/APC contact, the IS is stabilised, which occurs by co-ligation of TCR and CD28 or LFA-1. This co-ligation triggers an enhanced raft accumulation, resulting in increased Lck-activation and degradation and thereby sustained activation of tyrosine-phosphorylation (38). Co-stimulation by CD28 allows naive T cells to lower the threshold for T cell activation, whereas effector and memory T-cells require no CD28 engagement because of their higher amounts of raft microdomains in the plasma membrane (39). In contrast to effector/memory T cells, the raft ganglioside GM1 as well as Lck of naive T-cells are found to be stored in intracellular raft-like membranes (40). Therefore, to efficiently activate naive T cells, transduction of the intracellular raft-like membranes and the few plasma membrane rafts to the site of TCR triggering as well as CD80 and/or CD86 (APC)-CD28 (T-cell) interaction are required.

3.3. Rafts and pathogens

Although the beneficial list of raft-mediated functions in cells expands continuously, many pathogens are directly linked with lipid rafts or lipid raft-associated proteins (41, 42). Recent studies showed that a number of toxins, bacteria, protozoa and viruses bind to structures associated with membrane microdomains, e.g. endotoxins interact with GPI-anchored proteins (LPS-CD14 interaction) within the TLR-complex to trigger internalisation of the receptor and to activate mitogen-activated protein kinases (MAPKs) as well as cytokine production (43). Toxins – like cholera-toxin (CTx) – bind to the raft-associated ganglioside GM1 to enter the cell via this interaction. Furthermore rafts serve as interacting partners for pore forming toxins, and some bacteria use rafts to enter their host cells.

Recently it was also demonstrated that different viruses misuse rafts as entry and exit gateways (44). Not only influenza virus is entering and budding from the host-cell via rafts, but also measles virus (45), HIV and many other viruses (rev. in 1) infect their targets by interaction with the membrane microdomains. Scheiffele *et al.* (46) showed that different viruses (Fowl plague virus, FPV of the influenza family; vesicular stomatitis virus, VSV, Semliki forest virus, SFV) incorporate host-membrane lipids to a different extent into their envelope. The envelope of the FPV contained high amounts of detergent-insoluble complexes, which were absent from the other two virus types (VSV, SFV). It was shown earlier that specific viral glycoproteins, like influenza neuraminidase (NA) as well as hemagglutinin (HA) are localized in rafts. Efficient HA transport to the cell surface required cholesterol and the viral protein remained associated with rafts at the plasma membrane (47).

4. HIV AND RAFTS

HIV encodes only a small set of functional genes. Although the retroviral genes drive the replication cycle, recent studies revealed that in numerous phases of the HIV life cycle host cell proteins and lipids play a critical role. Lipid rafts in the cell membranes are abused by HIV in nearly all steps of its life cycle: passage through mucosal tissues (48), infection of permissive cells (49), transformation of signaling mechanisms of the host cell for efficient replication and immune evasion (50), exit of host cells (41), or entrance into the vascular system of the host (51).

4.1. Attachment and Entry

The modulation via rafts starts at the entry of HIV into the host cell by a high affinity interaction with two host-cell derived transmembrane receptors, CD4 and either CXCR4 (syncytium-inducing, T [T cell]-tropic, X4 HIV-1 strains) or CCR5 (non-syncytium-inducing, M [macrophage]-tropic, R5 HIV-1 strains) (52). HIV-1 attachment and entry into the target cell comprises multiple intermolecular interactions at the plasma membrane occurring sequentially. Firstly, HIV-1 interacts with CD4, which is thought to trigger conformational changes in gp120 to expose the chemokine receptor binding site. The subsequent interaction of the CD4-gp120 complex with the appropriate chemokine receptor results in gp41 conformational changes initiating the membrane fusion and infection process (53, 54). As both HIV-1 receptors (CD4, chemokine receptor) are suggested to localize preferentially in lipid rafts (55), rafts drive gp120/gp41, CD4, and the appropriate chemokine co-receptor into a membrane fusion complex (Figure 1).

Other molecules not directly involved in the HIV entry events may participate and facilitate the attachment process of the virus to its target (Figure 1). Additionally, defined glycosphingolipids of the host cell (Gb3, GM1), which are involved in gp120 interaction, are found in membrane microdomains and accessory molecules such as CD28 and CD44 may also promote HIV infection (38, 56) (Figure 1).

The C-type lectin DC-SIGN (CD209), which is abundantly expressed on immature dendritic cells (iDCs), functions as efficient binding and internalisation receptor for different viruses including HIV, CMV (57), Ebola (58), HCV (59), or other microorganisms (60-62). Non-opsonised HIV interacts via its envelope glycoprotein gp120 with DC-SIGN (63) (Figure 1). Recently, Cambi *et al.* (64) showed that DC-SIGN is distributed in well-organised microdomains on iDCs, is forming multi-protein assemblies on the plasma membrane and its binding capacity is partially affected by cholesterol extraction. They proposed the lipid raft-associated DC-SIGN molecules as efficient docking sites for HIV to invade the host.

In addition to DC-SIGN, other pathogen-capturing receptors such as FcRs and CRs were shown to localise in lipid rafts. Therefore, HIV, which is opsonised with antibodies and/or complement fragments *in vivo*, may bind to these receptors expressed on host cells, such as dendritic cells (65) or B cells (66) (Figure 1). This interaction mediates either an enhanced infection of the CR-/FcR-expressing cells in *cis* as shown for dendritic cells (65, 67) or also facilitates transfer of the bound opsonised particles to HIV-susceptible cells as shown for B cells (66). The raft-dependency of these interactions needs to be analysed in this context.

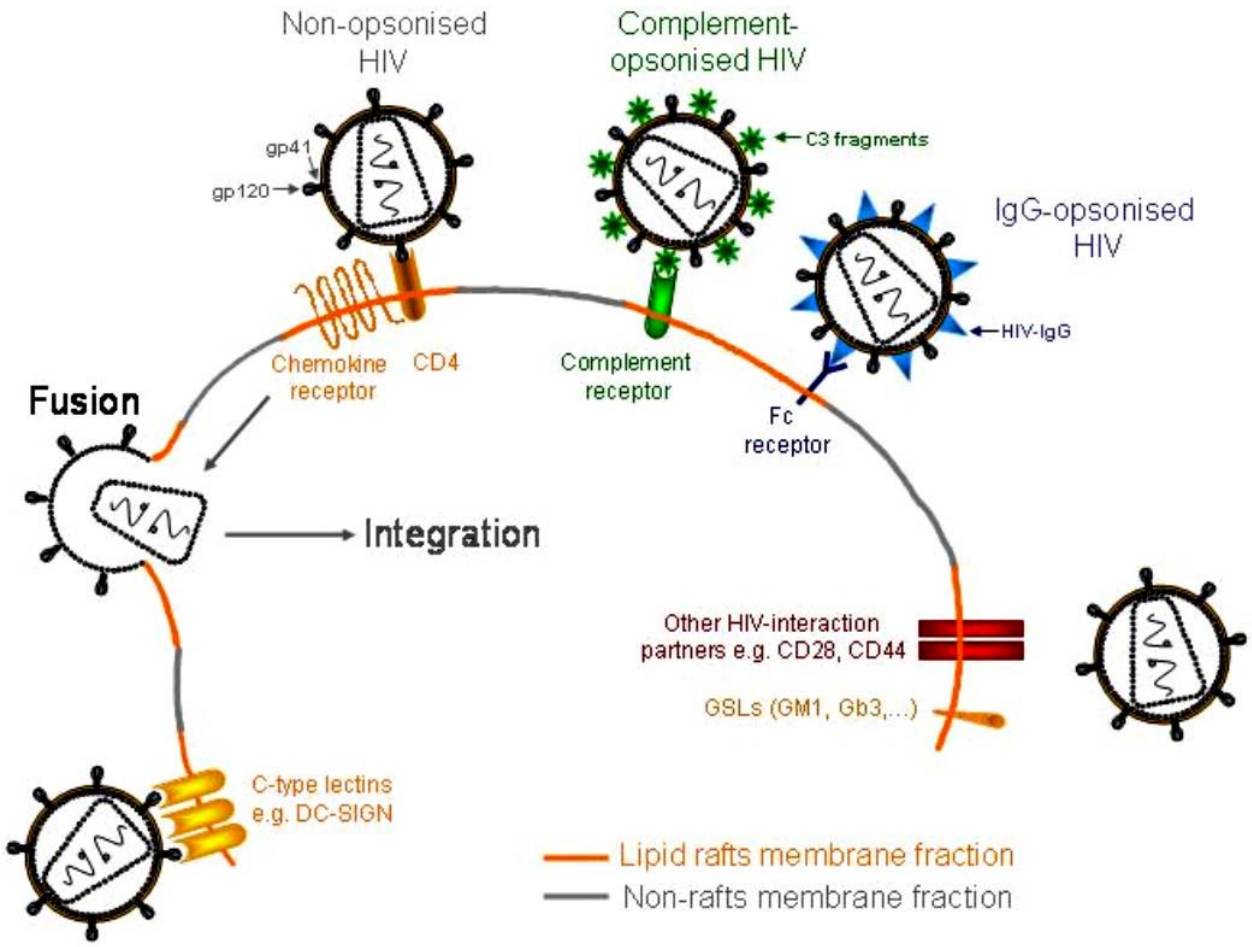

Figure 1. Initial binding of HIV to the host cell occurs mainly via lipid raft-associated molecules. Non-opsonised HIV can interact with the host cell via C-type lectins (e.g. DC-SIGN), CD28, CD44, GSLs (e.g. GM1, Gb3, etc.) or via CD4 followed by interaction with the appropriate chemokine receptor (CCR5, CXCR4), which leads to conformational changes in gp120 and gp41, which triggers the fusion reaction. When HIV is opsonised with complement fragments and/or HIV-specific IgGs, the virus can additionally bind to complement receptors (CRs) or Fcγ receptors, which were also shown to localise in lipid rafts.

Gummuluru *et al.* (68) used competitive inhibitors of HIV attachment to assign the contribution of the various receptors on DCs such as CD4, DC-SIGN, mannose receptor (MR) or heparin sulphate proteoglycans (HSPGs) with respect to HIV binding, DC infection and consecutive transfer to $CD4^+$ T cells. They found that DC-SIGN-, HSPG- and MR-independent mechanisms for HIV attachment and internalisation do exist and that the virus is efficiently transmitted to susceptible T cells although these receptors were inhibited. HIV binding, internalisation and transmission were dependent on lipid raft integrity, as the attachment of virus to DCs was drastically reduced by pre-treatment of DCs with raft-depleting reagents methyl-β-cyclodextrin or fillipin III. Thus, effective HIV binding and internalisation (in)to DCs and transmission from DCs to T cells seem to involve a multiplicity of different factors, that can at least in part substitute each other.

Furthermore, incorporation of host-derived proteins with cell-signaling capabilities into the viral membrane might have a number of consequences on adhesion of the virus as well as on viral entry, infectivity and pathogenesis.

The attachment and fusion process are not strictly dependent on the integrity of viral particles, as also isolated HIV proteins were shown to enter cells via rafts. The Tat protein of HIV-1 and HIV-2 is essential for transcription of viral genes, for viral replication, and acts as transcriptional activator of the integrated provirus. Besides its gene-regulating capacity, extracellular Tat was found to be able to enter cells in a heparin-dependent fashion, to traffic between cells and to be present in a number of cellular fractions (69, 70). Internalisation of Tat fusion proteins was shown to occur through caveolar endocytosis descending from lipid rafts, and not by clathrin-coated pit formation (rev. in 71). This internalisation route might be helpful for Tat to escape lysosomal degradation, to reach the Golgi apparatus, to be then transported to the ER and exported from the ER into the cytoplasm. The exact internalisation route was shown for choleratoxin B and has to be resolved in detail for Tat. Tat could also bind to virions budded from

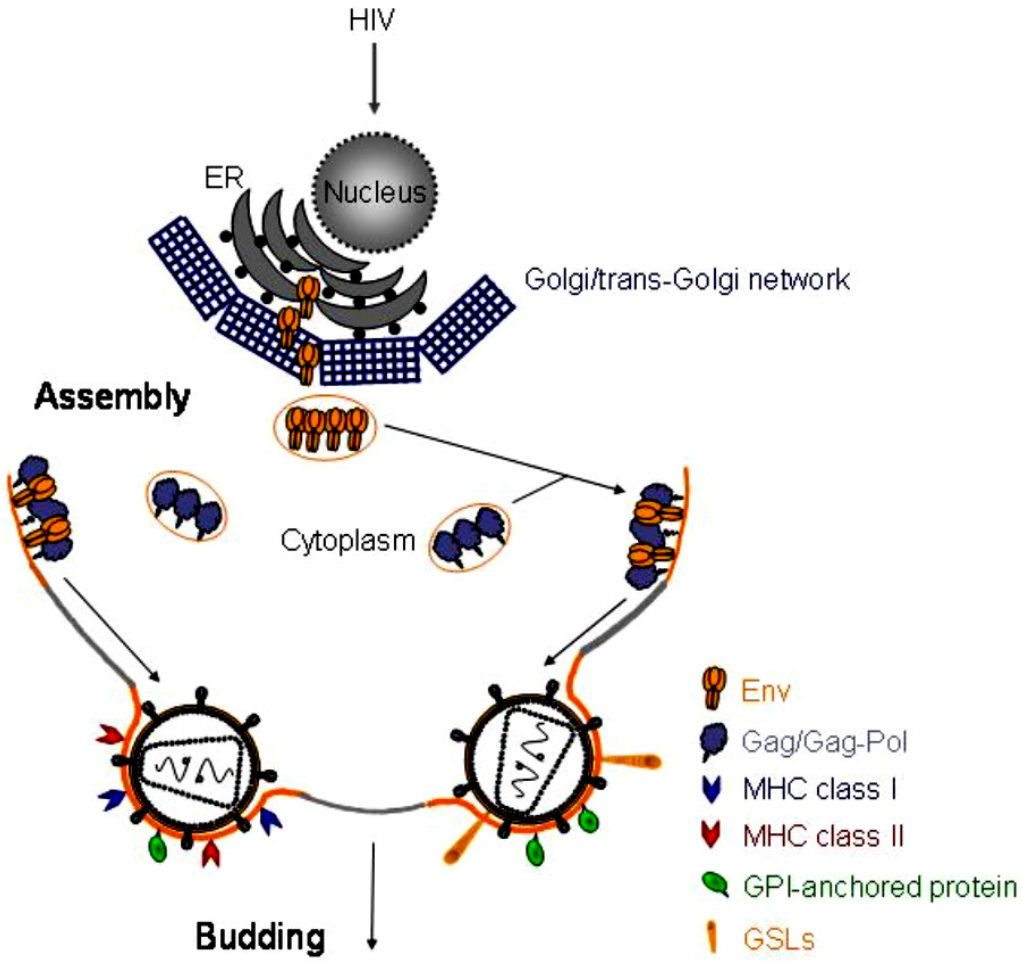

Figure 2. HIV abuses the lipid trafficking network for efficient replication. Env is synthesised in the ER and transported to the *trans*-Golgi network. Gag/Gag-Pol, is produced in the cytoplasm. Env and Gag/Gag-Pol are proposed to form a putative intracellular assembly complex, which is described to have similar characteristics to lipid rafts. When budding from the host cell through lipid rafts, HIV can incorporate selective host cell molecules, such as complement regulators (GPI-anchored proteins e.g. CD59), while excluding others, which may negatively affect virus propagation and/or survival.

host cells through heparan-sulfate proteoglycans on their surface. These virions could then enter a rafts-dependent pathway of caveolar transcytosis, allowing them to cross mucosal and blood-brain barriers (71).

Drugs sequestering cholesterol or inhibiting glycosphingolipid synthesis were shown to prevent HIV infection *in vitro* and *in vivo* indicating a role of raft dynamics for the efficiency of virus entry (49, 72-74). Removing cholesterol inhibited HIV-induced syncytium formation, reduced and interferred with co-receptor expression and function (73, 75, 76), and rendered cells resistant to infection with X4- and R5-tropic HIV strains without loss in cell viability (72). Binding of virus to CD4 or other attachment molecules thereby seems to concentrate viral receptors on the cell surface due to induction of lateral diffusion and coalescence of rafts, to alter the conformation of these and/or to organise platforms for trafficking to the appropriate compartment. Del Real *et al* (77) demonstrated that CD4 mutants targeting to non-raft fractions resulted in a CD4 receptor with highly diminished capacity for HIV entry or Lck signaling, despite similar binding of gp120 to this mutant compared to wild-type CD4 localised to lipid rafts.

Very recently controversial studies proposed that the receptors for HIV do not need to be localised in rafts, thus raising the question about the necessity of lipid rafts for ´productive entry´ of HIV into $CD4^+$ T cells (78). Popik and Alce (79) have shown that despite the preferential localisation of CD4 to lipid rafts, a CD4 receptor mutant excluded from raft microdomains also supported entry of HIV. They proposed that for virus entry CD4 raft localisation is not a requirement, but maybe post-binding fusion steps need association to lipid rafts.

4.2. Assembly and release

HIV or SIV particles were not only shown to attach and enter, but also to assemble and bud through lipid rafts (41) (Figure 2). The selective budding of HIV or SIV

allows on the one hand specific exclusion of host membrane proteins, which could negatively affect virus propagation and/or survival, and on the other hand the acquisition of specific host-cell membrane associated proteins and lipids into the virion that are able to deregulate cellular and/or humoral immune responses.

As shown in T cells by confocal imaging microscopy, the HIV core protein Gag and viral envelope proteins co-localise with lipid raft markers on the surface of infected cells, demonstrating that assembly and budding occur at sites of these membrane microdomains (41, 80). During assembly of HIV, viral proteins have to be transported from the cytoplasm to the plasma membrane and the transfer of HIV-Gag complexes to the plasma membrane for budding is suggested to be driven by multimerisation of Gag (81, 82). HIV-Env, that is synthesised in the ER and transported to the *trans*-Golgi network, together with Gag/Gag-Pol, which is produced in the cytoplasm, are proposed to form a putative intracellular assembly complex. This complex is described to have similar characteristics as lipid rafts, suggesting that HIV exploits the lipid trafficking network for efficient replication and reaching the plasma membrane (83, 84).

When HIV is budding from infected lymphocytes, host-cell associated, raft-localised molecules such as the complement regulatory, GPI-anchored proteins CD55 and CD59, MHC class I or class II, GM1 or FcR are incorporated, whereas the non-raft protein CD45 is excluded (41, 50, 85-88). This suggests that HIV is released selectively from lipid rafts. The incorporated host-associated molecules (HAMs) remain functionally active on the virus surface (88-90). They can protect the virus against the immune system or mediate attachment of HIV to their targets (65, 66, 91-94). Additionally, high amounts of cholesterol and sphingomyelin were detected on virions (95, 96). Graham *et al.* (97) showed that cell-free HIV and SIV virions were inactivated in a dose-dependent way by ß-cyclodextrin. They detected that viral membranes were permeabilised by ß-cyclodextrin treatment, resulting in damage of mature Gag proteins without loss of Env glycoproteins. Also reverse transcriptase, integrase, and viral RNA were absent in SIV virions, while integrase seemed only slightly decreased in HIV. The RNA, RT, matrix, and nucleocapsid proteins were retained in HIV virions upon ß-cyclodextrin treatment, but to a much lower degree (97). Furthermore Graham *et al.* (97) could show that intracellular associated HAMs – such as actin or moesin – as well as extracellular HAMs – such as MHC class I or class II – remained associated with the ß-cyclodextrin-treated virions. Thus, it seems that despite disruptions in virion membrane integrity, the overall structure of the virion can be maintained.

Not only Gag, but also the cytoplasmic domain of gp41 seems to be associated with the assembly of virions at the plasma membrane. gp41 contains two cysteine residues (C764, C837) in its cytoplasmic tail that are targets for palmitoylation. One of the cysteine residues (C764) in gp41 may support anchoring of this region to the plasma membrane. The precise function of this cytoplasmic domain needs to be determined, but it was reported to be essential for envelope association with lipid rafts and assembly of budding viral particles (98). Additionally, studies of HIV released from primary macrophages demonstrated the incorporation of lipid raft-associated proteins such as the GPI-anchored protein CD55 (99). Nguyen *et al.* (99) furthermore showed that also the tyrosine phosphatase CD45, which is specifically excluded from T cell rafts, was found to be associated with rafts in macrophages, thus suggesting that rafts on macrophages display different characteristics than those on T cells. Virions budding from macrophages incorporated amongst others raft-associated proteins such as CD36, CD55, MHC class I and MHC class II, or CD63 and Lamp-2, but excluded macrophage raft-associated molecules like CD45 and CD14. The lack of CD45 and the GPI-anchored protein CD14 gave rise to the presumption that HIV budding from macrophages would proceed by a different mechanism than via plasma membrane rafts. Macrophage-derived HIV particles showed a host molecule incorporation pattern characteristic for exosomes. Exosomes contain membrane proteins normally expressed in late endosomes such as CD63, Lamp-2, MHC molecules or CD86. Thus, it is assumed that HIV deriving from macrophages leaves the cells via an exosome-release pathway (Trojan exosome hypothesis [100]). The issue, whether lipid rafts display platforms for HIV-1 assembly and budding, remains controversial. Initially, raft localisation of Gag was based on the observation that high amounts of the total intracellular Gag were resistant to Triton X-100 extraction (41, 81, 82). Sol-Foulon *et al.* opposed the localisation of Gag in ´classical´ rafts due to higher densities of Gag protein assemblies and failure to incorporate traditional lipid raft components (101).

5. SIGNALING

Concomitant with optimisation of virus entry, replication and budding, HIV can use raft signaling to efficiently evade recognition and killing of infected cells (50). The ability of each participant of the HIV attachment and fusion process to initiate various signaling pathways and also the amount of signaling enzymes and adaptors in rafts addresses the issue, that rafts are used by the virus to subvert immune cell signaling. HIV has evolved various mechanisms to exploit its host, including the interruption and activation of signal transduction pathways not only in T cells (102, 103) but also in DCs (104) and macrophages (105, 106). This section will provide an overview of the relatively limited data investigating HIV-induced signaling via rafts.

5.1. Env: gp120

The capacity of the HIV envelope glycoprotein gp120 to induce intracellular signals is suggested to contribute to HIV pathogenesis. gp120 misappropriates the signaling function of the CD4-p56^{lck} complex to act on CD4 T cell signaling (Figure 3A). Additionally, ligation of gp120 with the CD4-chemokine receptor complex was shown to activate a variety of intracellular signaling pathways including mobilisation of intracellular Ca^{2+} (107), phosphorylation and thus activation of many cellular

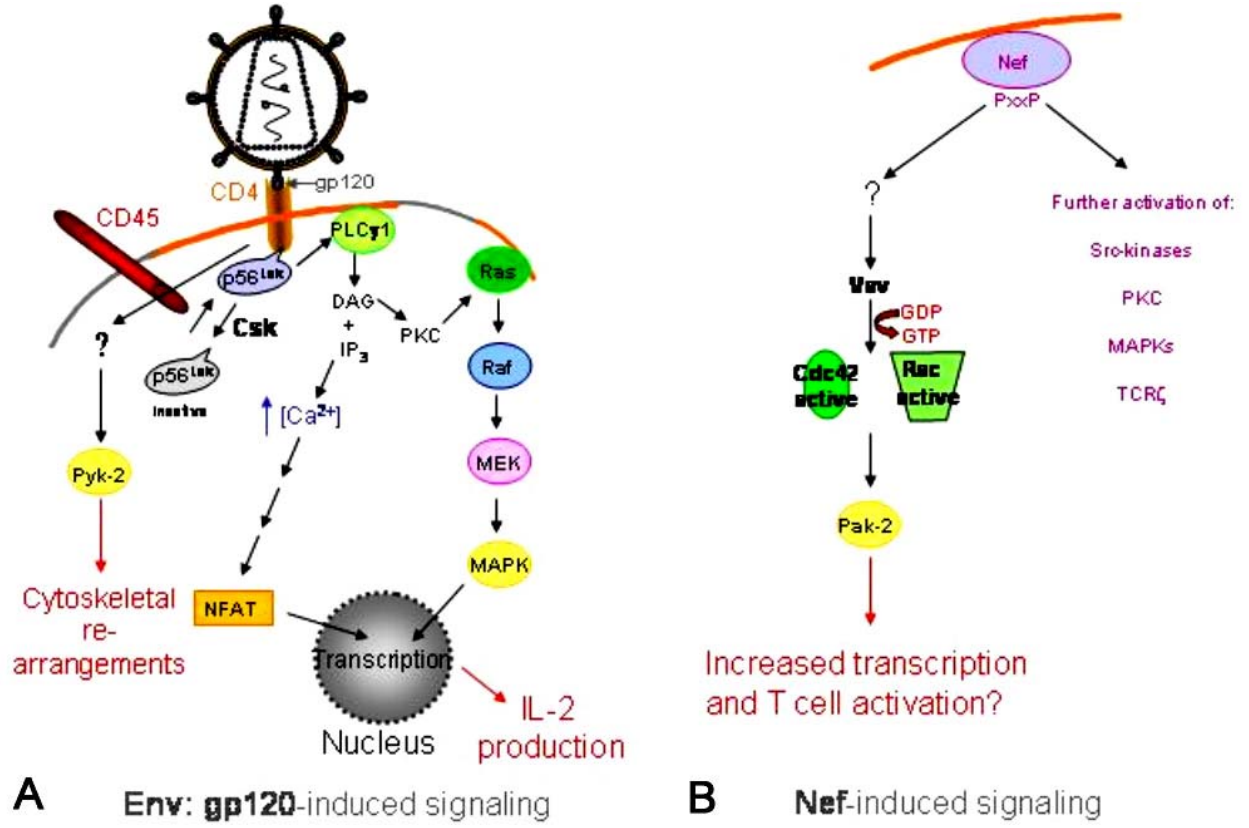

Figure 3. HIV induces signaling via rafts. Schematic overview of (3A) gp120-triggered signaling events and (3B) Nef-mediated signaling are shown.

molecules such as PKC (108), Zap-70, Ras and MAPKs (109, 110). Some of these molecules are likely required for activation of NF-κB and AP-1 transcription factors (110). The gp120-induced activation of MAPKs was dependent on the ganglioside GM1, thus indicating receptor binding via membrane microdomains (111). Kinet *et al.* demonstrated a gp120-induced recruitment of MAPKs under certain activation conditions of primary lymphocytes, thus revealing a strategy of different modulation of quiescent and activated T cells via gp120. Short-term stimulation of primary lymphocytes with gp120 caused phosphorylation of ERK1/2 and Pyk2 and these activated signaling molecules distributed in lipid rafts (3). Pyk2 is a member of focal adhesion PTKs involved in cytoskeletal re-arrangements by associating with paxillin or other cytoskeletal proteins. Also in human macrophages and smooth muscle cells HIV env was shown to activate the MAPK signaling module or focal adhesion tyrosine kinases (112, 113). It was furthermore shown in human primary B cells that gp120 inhibits B cell chemotaxis to CXCL12, CCL20 and CCL21 in a CXCR4 or CCR5- and lipid rafts-dependent fashion via p38 MAPK activation (114).

5.2. Nef

Nef is an important virulence factor of primate lentiviruses. *In vivo* Nef enhances viral infectivity, accelerates disease progression, modulates surface expression of different cellular proteins including CD4 and MHC class I, and affects signal transduction pathways (Figure 3B). The exact molecular mechanisms of Nef to alter host cell signaling are not fully solved yet, but key motifs required for Nef signaling are the myristoylation signal and the proline-rich region of Nef (115). The highly conserved proline-rich motif (PxxP) of Nef was shown to bind the SH3 domain of a variety of raft-associated molecules including Src family kinases (Hck, Fyn, Lck, Lyn), protein kinase C (PKC), MAPKs, Pak2, TCRζ, or Vav (116-118). Furthermore, Nef activated raft-associated molecules such as Lck or Lyn even in the absence of stimulation (50) and the MAPK pathway in primary T cells (119).

Additionally, Nef-induced prevention of apoptosis of infected cells and promotion of death of activated bystander CD8 T cells were described (rev. by 120, 121). Nef mediated its anti-apoptotic effects by phosphorylation of Bad (122) and by inhibiting apoptosis signaling regulating kinase 1 (ASK1) (123).

When T cells were activated by MHC-bound Ag, 5-10% of the entire cellular Nef pools were found localised in lipid rafts within minutes after formation of the immunological synapse (124). Nef was furthermore shown to interact with p21-activated kinase 2 (Pak-2) in lipid rafts (125), which may result in increased frequency of cells expressing transcriptionally active forms of NF-κB and

NFAT and increased T cell activation (124). The interaction of Nef with Pak-2 was mediated by the upstream effectors of Pak-2, Cdc42 and Rac, since Nef was shown to activate these small Rho-GTPases via Vav (126, 127). Activation of Pak-2 by Cdc42 and Rac occurred in rafts and depended on raft integrity. To elucidate the initial trigger of the Nef-mediated signaling pathway, Simmons *et al* (128) performed proteomic analyses and found that in Nef-expressing $CD4^+$ cells the E2 ubiquitin-conjugating enzyme UbcH7 was absent from rafts. UbcH7 is implicated in negative regulation of T cell signaling (129). Exclusion of UbcH7 from rafts would result in accumulation of tyrosine-phosphorylated Vav leading to increased Cdc42 activity. They showed that a ternary complex between Cdc42, the Pak-interactive exchange factor p85Cool1/βPix and c-Cbl seemed to be responsible for the loss of UbcH7 in lipid rafts and the subsequent loss of ubiquitination of Vav. Nef-induced, constitutive active Cdc42 localised at the plasma membrane could be required to efficiently form the virological synapse necessary for efficient cell-cell transfer of virus (130, 131).

Another study by Djordevich *et al* (132) proposed that the myristoylated Nef would prime resting T cells by increasing levels of signaling molecules within rafts, and that in turn TCR activation is enhanced by the capacity of Nef to promote raft fusion.

Again, there is a controversy whether rafts play a key role for the observed Nef-mediated effects. Sol-Foulon *et al.* (101) proposed that the effects of Nef on trafficking of cellular proteins and on viral replication were independent of lipid rafts.

6. CONCLUDING REMARKS

Immune cells respond to their environment through a large number of signaling receptors and dynamic spatial re-organisation of the receptors. Such re-organisation seems to be important to optimise and control cellular signaling and duration of cell activation (133). All molecules essential for HIV propagation are either concentrated in membrane microdomains or recruited to the virological synapse in an active way on both, the HIV-infected cell as well as the target cell. The re-organisation to the virological synapse between HIV-infected antigen-presenting cells such as dendritic cells and $CD4^+$ T cells was visualised by McDonald *et al.* (130) using live imaging microscopy. Not only the re-organisation of signaling molecules and receptors to lipid rafts upon activation, but also the capacity of several proteins to signal from rafts as well as non-raft domains with completely different qualitative and quantitative outcomes, confers to the complexity of the membrane/signalosome. HIV seems to misuse these cellular mechanisms to efficiently enter the host cells and replicate. Several of the proteins encoded by HIV contribute to the modulation of mitogenic and apoptotic pathways in various hematopoietic and nonhematopoietic cell types. It is obvious that gp120 and Nef are not the sole actors in these signaling processes. In as much additional HIV-1 regulatory proteins could also induce multiple signaling responses needs to be investigated. A further open question is, in as much specific disruption of HIV-lipid raft interactions may be of therapeutic interest.

7. ACKNOWLEDGEMENTS

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