Thematic Review Series: Biology of Lipid Rafts

Lipid rafts and pathogens: the art of deception and exploitation

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Abstract   Lipid rafts, solid regions of the plasma membrane enriched in cholesterol and glycosphingolipids, are essential parts of a cell. Functionally, lipid rafts present a platform that facilitates interaction of cells with the outside world. However, the unique properties of lipid rafts required to fulfill this function at the same time make them susceptible to exploitation by pathogens. Many steps of pathogen interaction with host cells, and sometimes all steps within the entire lifecycle of various pathogens, rely on host lipid rafts. Such steps as binding of pathogens to the host cells, invasion of intracellular parasites into the cell, the intracellular dwelling of parasites, microbial assembly and exit from the host cell, and microbe transfer from one cell to another all involve lipid rafts. Interaction also includes modification of lipid rafts in host cells, inflicted by pathogens from both inside and outside the cell, through contact or remotely, to advance pathogen replication, to utilize cellular resources, and/or to mitigate immune response. Here, we provide a systematic overview of how and why pathogens interact with and exploit host lipid rafts, as well as the consequences of this interaction for the host, locally and systemically, and for the microbe. We also raise the possibility of modulation of lipid rafts as a therapeutic approach against a variety of infectious agents.—Bukrinsky, M. I., N. Mukhamedova, and D. Sviridov. **Lipid rafts and pathogens: the art of deception and exploitation.** *J. Lipid Res.* 2020. **61:** 601–610.
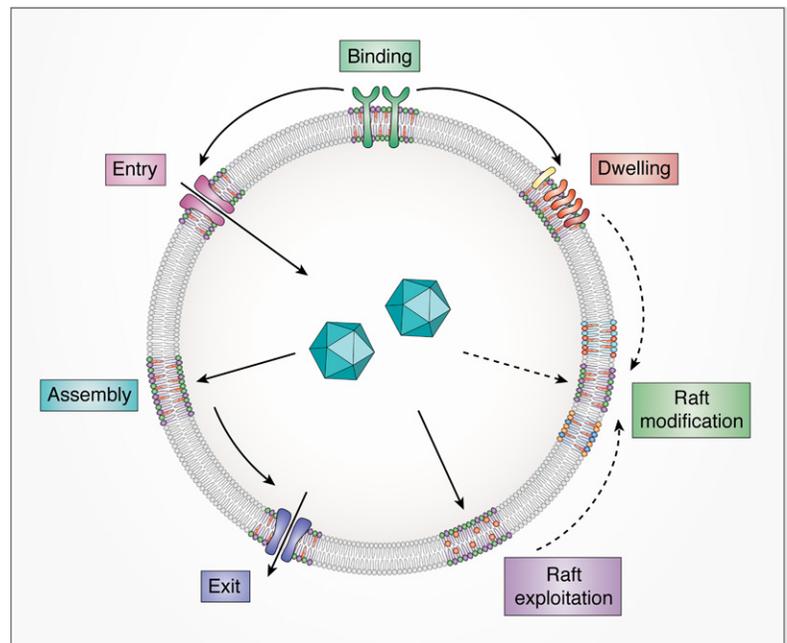
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Lipid rafts are dynamic nanometer-size scale regions of the plasma membrane enriched in cholesterol and glycosphingolipids. They are characterized by distinct lipid and protein composition and tight ordered lipid packing; their structure is determined by the interaction between resident

Abbreviations: ESCRT, endosomal sorting complex required for transport; EV, extracellular vesicle; HA, hemagglutinin; HIV, human immunodeficiency virus; IAV, influenza A virus; PV, parasitophorous vacuole; SA, sialic acid.

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lipids and proteins. Structurally, they are solid bits immersed into the fluid environment of the membrane. Functionally, they host most of the plasma membrane signaling, as well as exo- and endocytosis machineries, forming the functional platforms for the interaction of cells with the outside world (1). Lipid rafts are structurally and functionally heterogeneous, within the cells and between the cells, both spatially and temporally. The functional heterogeneity of the rafts is governed by a repertoire of proteins in a particular subfraction of the rafts, which is determined by a combination of proteins' raft-targeting motifs and posttranslational modifications (2).

From the microbe's point of view, a lipid raft on the membrane of the host cell is an island with an airport in the middle of the ocean. Lipid rafts are characterized by the presence of a high concentration of outward-looking protein molecules providing a guidance system and a landing gear to ensure precise and safe delivery of a microbe to the right terminal in a correct position. Lipid rafts host the endo- and exocytosis machinery, an automated loading/unloading facility connected to a railway effectively transporting cargo to and from the intracellular locations. Although there is a border protection force and customs trying to prevent unlawful entry and smuggling, pathogens are rigorously selected specialists in deception and win on many occasions. The biggest problem for the pathogens is not the host defense, but the physical size and dynamic nature of the runway: rafts are often too small for larger microbes. In response to this limitation, microbes developed a capacity to modify the airfield or themselves according to requirements.

Pathogens of different taxa exploit host lipid rafts to advance the infection. The most prolific users are intracellular parasites [viruses, intracellular bacteria, protozoa, and fungi (3)], and the lifecycle of these microbes critically depends on host lipid rafts. Microbes that do not enter the host cell also take advantage of the lipid rafts to gain access to the cellular molecules not available to a microbe through its own biosynthesis. Microorganisms can deliberately modify lipid rafts either to adapt them according to the pathogen's requirements or to inactivate many immune defense pathways relying on lipid rafts. By modifying lipid rafts, however, microbes do significant collateral damage to the host causing numerous comorbidities. It is not an intension of this review to provide a complete list of microbes interacting with host lipid rafts, several comprehensive reviews on this topic have been published recently (3–5). Instead, we provide a systematic overview of the principles of interaction of microbes with the host lipid rafts (summarized in **Fig. 1**) and illustrate these principles using a selection of the most interesting, from our point of view, examples.

BINDING

Initial physical interaction of a microbe with a host cell is a key first step often determining what happens next, and there is no better place to gain control of the host cell than through initial contact with the lipid rafts. Most microbes are selective in their choice of the target cells, and the presence of surface receptors specific for a particular cell type (receptor "signature") concentrated in a confined space, lipid rafts, determines whether to bind or not. A high concentration of the receptors in one place also fulfils a frequent requirement that more than one connection needs to be made to firmly attach the pathogen to the host and guide it to the right place. In addition, close proximity of the rafts to endo- and exocytosis machinery, which is required for internalization of pathogens, facilitates the next steps of infection.

Human immunodeficiency virus (HIV-1) is a well-studied example of a virus exploiting lipid rafts for binding to the target cell. The main HIV-1 receptor, CD4, and coreceptor, CCR5, localize preferentially to lipid rafts (6, 7); whereas lipid raft localization of another alternative coreceptor, CXCR4. is only partial (8, 9). The lipid environment facilitates controlled gp120-dependent clustering of CD4 and coreceptors during virus-cell interaction, which is required for efficient fusion (10–12).

Another example of a medically important pathogenic virus that uses lipid raft-localized molecules for binding is influenza A virus (IAV). The IAV envelope protein, hemagglutinin (HA), binds to sialic acids (SAs), which are a component of gangliosides that decorate many lipid raft proteins (13). Because most SA-decorated proteins that bind the virus cannot trigger IAV endocytosis on their own, the virus has to roll over many binding sites until it attaches to a specific entry receptor protein, such as the killer-activating proteins NKp44 and NKp46, the epidermal growth factor receptor, or voltage-dependent Ca^{2+} channel $Ca_v1.2$ (14–17). The rollover is facilitated by another IAV envelope protein, neuraminidase, which degrades SA near the binding site allowing the virus to move to another location (18). This process is made much more efficient by concentration of SA-decorated proteins in the lipid rafts.

Bacterial and protozoan parasites also benefit from using lipid rafts as a binding platform. A problem for these pathogens in taking advantage of the rafts is that these microorganisms are usually bigger than the rafts, so microbes developed various strategies to deal with this limitation. For example, the lifecycle of the *Chlamydia* species is dimorphic and the infectious form of the pathogen, the elementary body, is smaller in size (19), allowing many, although not all, species to bind to lipid rafts (20). The exact host receptor driving this binding is not known and the process might occur through binding of fibroblast growth factor 2 to the microbe and subsequent binding to the fibroblast growth factor receptor (21). *Shigella* species attach to the rafts of intestinal cells, and the host receptor appears to be the raft-associated CD44 (22). Trophozoites of *Plasmodium falciparum* enter red blood cells through lipid rafts sequentially using multiple receptors for firm attachment and taxiing, but then disrupt the rafts behind them when they are inside (23, 24).

DWELLING

Prions are a unique example of a pathogen where the entire pathogenic activity takes place in lipid rafts. Prions
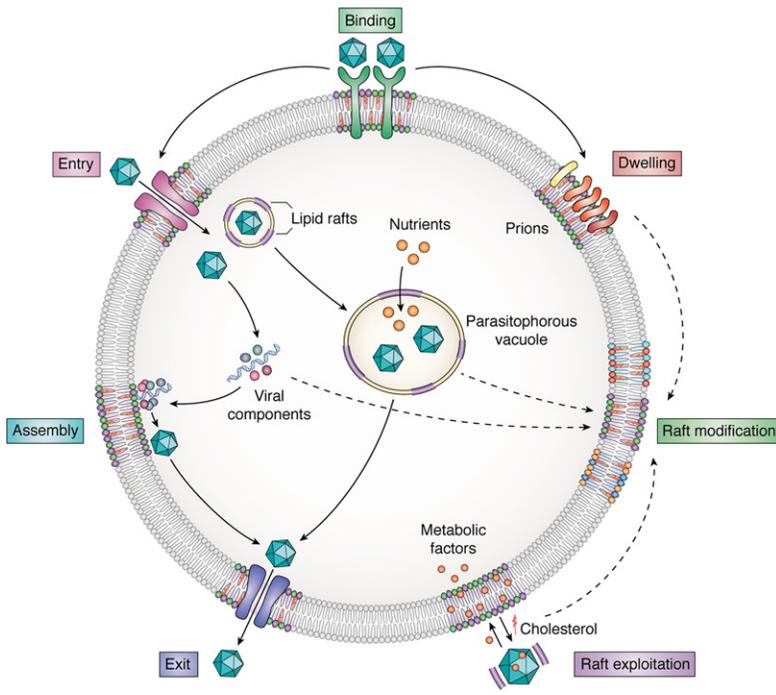
Fig. 1. Overview of the interaction of pathogens with host lipid rafts. Various pathogens use a high concentration of outward looking receptors in lipid rafts as a binding site, and raft-associated endocytosis machinery as a pathway to get inside of the host cell. Some intracellular parasites take rafts with them during internalization and use them to communicate with the host cell and to prevent maturation or fusion of microbe-containing endocytic vesicles (e.g., PV) with lysosomes. Prions find a high concentration of physiologically folded proteins in rafts converting them into pathological form. Many pathogens modify lipid rafts, acting both from inside and from outside the cells, both through binding to rafts and remotely, through releasing raft-modifying factors. Viruses often use lipid rafts as a platform to facilitate self-assembly, and various pathogens exploit involvement of lipid rafts in exocytosis to get out. Details are in the text.

are the most unusual infectious agents: they do not have any genetic material and likely consist of just one protein, a prion, in a misfolded conformation, PrP^{Sc} (25). Physiologically folded prion, PrP^C, is a ubiquitous host protein located in lipid rafts (26). When pathogenic PrP^{Sc} interacts with host PrP^C, it initiates a chain reaction of misfolding converting host PrP^C into PrP^{Sc}. A high abundance of the rafts where PrP^C is concentrated in a stable environment sheltered from endocytosis (27) is a prerequisite for nucleation of the misfolding cascade. Furthermore, accumulation of PrP^{Sc}s in rafts modifies them by displacing ABCA1, reducing cholesterol efflux, and promoting new raft formation, thus adding another dimension to the vicious cycle of prion diseases (28). It should be noted, however, that internalization of prions has been described, although it is not clear if this is part of pathogenesis or a defense mechanism (29).

ENTRY

Usually, following binding to lipid rafts, a pathogen invades the target cell. An array of mechanisms is used by pathogens for this step, and often the same pathogen has various tools at its disposal, using and sometimes mixing pathways depending on the type of host cell and environmental circumstances. The route of entry often utilizes lipid rafts, although this is not the only option and pathogens also use raft-independent pathways, predominantly or optionally (30).

The most straightforward way to enter a cell is to make a pore in the plasma membrane. Making a pore is easier in a solid environment where "a hole" can be sustained for at least some time, i.e., in a lipid raft. However, making holes in the rafts is associated with two potential problems for the

pathogen. First, this may irreversibly damage the host cell, as happens during infection with *Vibrio cholera* (31), leading to rapid cell death. Second, after entering through a pore, the microbe gets into a vast intracellular space far away from the intended destination. Therefore, many microbes employ a modified approach where creating a pore is part of a complex fusion event, which can take place either at the plasma membrane or inside the cells at the membrane of endosomes. In general, most enveloped viruses use lipid rafts, either on the plasma or endosomal membrane, for entry, highlighting a conundrum, as ordered lipid domains, including lipid rafts, are thermodynamically unfavorable for membrane fusion. Nevertheless, fusion between viral and cellular membranes depends on cholesterol and sphingolipids (32–34), which are concentrated in lipid rafts. Possible solutions for this conundrum include cholesterol influencing fusion by modifying the conformation of the transmembrane domain of the fusion protein, as has been suggested for SNARE-mediated fusion (35) or gp41 (36). Further, because of its effective molecular shape (relatively small in cross-section polar group and large hydrophobic part), cholesterol is expected to, and indeed does promote the bending of the lipid monolayer into early hemi-fusion intermediates (37, 38). Recent studies provided a possible mechanism for the role of lipid rafts in fusion. Yang and colleagues demonstrated that line tension at boundaries between liquid-ordered (lipid rafts) and liquid-disordered (non-raft regions) membrane domains driven by hydrophobic mismatch contributes energy to drive HIV gp41-fusion peptide-mediated fusion (39, 40). Interestingly, the amount of energy contributed to fusion from line tension in a model system depends on the size of fusing vesicles, and in the case of HIV infection would favor fusion with larger raft domains (40). This consideration is consistent with coalescence of the lipid rafts in activated

CD4+ T cells correlating with susceptibility to HIV infection (41, 42). It also underscores the importance of the lipid raft dynamics for the function of these domains as entry ports for invading viruses.

Detailed mechanisms of the virus-cell fusion process have been extensively reviewed (43, 44). HIV has long served as an example of a virus fusing at neutral pH with the plasma membrane (45), although direct visualization of HIV-1 fusion at the endosomal membranes has been also reported (46). While HIV fusion with the endosomal membranes has been considered a nonproductive pathway (47), a recent study suggested that HIV-1 enters T cells exclusively via endocytosis (48). This view has been challenged in several other reports (49–51) and remains controversial. It is likely that HIV can use alternative locations for fusion depending on target cell type and environment. Nevertheless, most enveloped viruses fuse with the endosomal membrane after endocytosis, and lipid rafts likely play a role in endosomal membranes similar to that in plasma membranes. In addition to their role in fusion, lipid rafts on endosomal membranes may prevent or delay maturation of the endosomes into lysosomes (52), thus providing time for the fusion to take place.

Another classic example of a virus exploiting the endocytosis pathway for entry is the influenza virus (53). As discussed above, the virus preferentially binds to lipid rafts. However, the virus can enter the cell both via lipid raft-dependent and raft-independent clathrin-mediated endocytosis (54). The entry pathway likely depends on the type of infected cell and environment, but the mechanistic details behind the selection remain unknown. The fusion process is initiated by acidification of endosomal pH during internalization (55). Low pH triggers the conformational changes in viral HA protein, which drive the fusion process (56). Acidification of the endosomes also lowers the pH of the virion interior due to the passage of protons through the ion channel formed by the M2 protein; this acidification is required for release of the viral ribonucleoproteins into the cytoplasm of the infected cell (57). Interestingly, both HA and M2 proteins are targeted to lipid rafts (58, 59), underscoring the role of these domains in influenza virus fusion. Influenza is a good example of a pathogen exploiting both raft and non-raft parts of the membrane for entry.

Lipid rafts are essential for entry of a large number of other pathogenic viruses, including such deadly filoviruses as Ebola and Marburg. Disruption of lipid rafts by cholesterol-extracting agents (e.g., cyclodextrin) blocked entry of Ebola virus and Marbury virus invasion (60, 61). The molecular mechanisms underlying such dependence of filoviruses' entry on rafts are not fully understood. One of the identified receptors, folate receptor-α, is a GPI-anchored protein that localizes to rafts (62). Localization of two other receptors, TIM-1 and TIM-4 (63, 64), has not been characterized.

Escherichia coli K1, a cause of neonatal meningitis, enters the endothelial cells via macropinocytosis utilizing host pinocytosis machinery located in lipid rafts; integrity of the host lipid rafts is paramount for the invasion to occur (65). The same bacteria enter peripheral blood monocytes, macrophages and microglia using phagocytosis machinery, also located in lipid rafts. In monocytes and macrophages, the invasion results in propagation of the bacteria (66); however, in microglia the infection is aborted (67). Thus, entry via pinocytosis and phagocytosis, both involving lipid rafts, may lead to productive as well as abortive infection depending on a cell type.

On many occasions the exact mechanism of entry has not been established, although the role of lipid rafts was demonstrated. *Streptococcus pneumoniae* evades immunity by hiding in erythrocytes after entering the cells via lipid rafts, but the exact mechanism of invasion is not known (68). An interesting example is co-infection with *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, when the two infectious agents require each other to enter the epithelial cell; they utilize lipid rafts as an entry gate, but how this alliance works in not clear (69).

Fungi also use host lipid rafts to enter the cell. *Candida albicans* uses the phagocytosis machinery located in rafts to enter monocytes (70), but often fungi bring their own machinery for entry. *Histoplasma capsulatum* requires rafts to stabilize the microbe at the cell surface and trigger internalization (71). *Encephalitozoon cuniculi*, like many other microsporidia, enters cells by discharging a hollow tube followed by formation of a parasitophorous vacuole (PV). Because initial binding occurs in lipid rafts, the parasite uses rafts along with surrounding non-raft portions of the plasma membrane as building material for both the hollow tube and PV and likely uses rafts incorporated into a PV for transport of nutrients into the PV (72).

Yet another mechanism of entry (and also exit) relying on lipid rafts is formation of the virological synapses and nanotubes to transfer viruses from one cell to another via a cell-to-cell connection avoiding any contact with blood and systemic immune factors, such as antibodies. This form of transmission, sheltering the virus from interaction with antibodies, was described for HIV (73), cytomegalovirus (74), human T-lymphotropic virus-1 (75), and some other viruses (76, 77). Virological synapses are responsible for the transcytosis of HIV, where HIV is translocated across a barrier of epithelial cells, which normally are not infected by HIV, in order to reach and infect underlying target cells (78). Assembling intercellular contacts and nanotubes requires multiple elements to come and hold together in a limited ordered space. Rafts are ideally suited for this, and, at least for HIV, the key role of rafts in formation of virological synapse has been demonstrated (79).

INSIDE JOB

The important role of the host lipid rafts in the life of intracellular microbes does not end when a pathogen is inside, and several examples of pathogens exploiting rafts on the membrane of endosomes were described above. Generally, endosomal lipid rafts are used by pathogens in three ways: as a signal to drive endosomal vesicles away from lysosomes, as a communication channel between host and microbe, and as a platform for the microbe replication

and assembly. Many proteins determining the destination of an endosomal vesicle are raft proteins, and raft modification by a microbe before and during endocytosis is a way to hijack the pathway directing it away from phagolysosome fusion. An example is *Leishmania donovani*, which disrupts clustering of dynein in rafts preventing phagosome fusion with lysosomes (80). Another example is *Chlamydia trachomatis*, which binds to and internalizes through caveolae forming caveolin-coated endocytic vesicles. These vesicles acquire more caveolin from the Golgi, disguising the vesicles as exocytic vesicles in order to avoid fusion with lysosomes (52). Hepatitis C virus also relocalizes lipid rafts from the plasma membrane into the autophagosome; the resulting unique structure, "raft-autophagosome," is capable of hosting hepatitis C virus replication machinery (81).

Many intracellular parasites dwell inside the host cells in a PV or other forms of replicative inclusions to separate parasite's living quarters from the rest of the host cell. The external membrane of these inclusions is formed during endocytosis using fragments of the host plasma membrane, and as microbes often bind to and are internalized from lipid rafts, it contains elements of or entire host lipid rafts (82). These lipid rafts are then used to deliver essential nutrients into the vacuole through the pores (72) or utilizing raft-associated endocytosis machinery (83). Rafts, however, require maintenance with constant delivery of cholesterol and sphingomyelin, lipids that microbes are unable to synthesize. Microorganisms such as *Chlamydia* developed pathways to utilize cellular cholesterol, sphingomyelin, and ceramide to maintain mosaic structure of their inclusion membrane (84).

EXPLOITING AND MODIFYING LIPID RAFTS

Binding to, dwelling in, and invading through lipid rafts inevitably modify rafts as described above, but facilitation of the invasion is not the only benefit for a microbe rising from interaction with cellular lipid rafts. Two other gains are impaired immune response resulting from modification of lipid rafts and the possibility to exploit lipid rafts as an access point to a source of energy and/or building materials. Importantly, a microbe doesn't have to be inside the cell to achieve these gains. *Helicobacter pylori* is a good example: after attaching to lipid rafts in the gastric epithelium, it breaks down the rafts by removing cholesterol from the rafts and injecting a raft-modifying effector, CagA, and a toxin, VacA, into the cell (85). Reducing the abundance of lipid rafts blocks interferon γ and β signaling in gastric cells preventing cytokine release and allowing *H. pylori* to escape host inflammatory response (86). Apart from using host cholesterol to build its own membrane, *H. pylori* expresses a unique enzyme, cholesterol-α-glucosyltransferase, which converts excess cholesterol into cholesteryl glucoside (87). This, on the one hand, reduces toxicity of the extracted cholesterol and, on the other hand, modifies lipid rafts in macrophages inducing autophagy and increasing bacterial survival (88). *Borrelia burgdorferi* is a cholesterol auxotroph; it binds to the host cells and extracts cholesterol from host rafts, either directly or through promoting formation of extracellular vesicles (EVs), to build its own lipid rafts (89, 90).

There are many examples when interaction of pathogens with host cells profoundly changes the properties of host lipid rafts. This applies to the rafts the pathogen interacts with, but often affects all rafts in the infected and even in uninfected cells. The benefits for the pathogen rise from the critical role rafts play in inflammation, immune signaling, and other forms of anti-infection defenses; modification of the host lipid rafts reduces the strength of these defenses. Furthermore, as illustrated below, raft modification may improve local and systemic conditions for expansion of the infection, e.g., providing additional and improved platforms for interaction of microbe with host cells. Often impaired defenses and better replication conditions are combined and intermix with the defense responses of the host, also involving rafts, resulting in a complex picture.

HIV increases the abundance of lipid rafts on infected and uninfected cells creating additional capacity for virus assembly and facilitating binding and infection by other HIV units (91–93). The virus also modifies raft properties, on the one hand, impairing phagocytosis, an element of the defense mechanism (91), but on the other hand, stimulating inflammation, another element of defense response. Stimulation of inflammation may reflect the defense response of the host, but may in fact facilitate the infection, as HIV preferentially targets activated CD4+ T cells for infection (94). To modify lipid rafts in uninfected cells, the virus unleashes Nef-containing EVs from infected cells, which impair activity of ABCA1 and inhibit cholesterol efflux in targeted cells, leading to increased abundance of lipid rafts (95). Remote manipulation of lipid rafts by EVs is also used by *Pseudomonas aeruginosa*, which modifies host lipid rafts not only after binding to them but also by packaging a swag of virulence factors into EVs (in the case of bacteria, known as outer membrane-derived vesicles). EVs released from the bacteria enter the host cell through lipid rafts, modify rafts as well as many other elements of cell metabolism, and create a favorable environment for subsequent infection (96). A different strategy for raft modification is used by cytomegalovirus. This virus does not use lipid rafts for entry, allowing it to employ a virus-derived pathway engaging viral protein US28 to enhance cholesterol efflux and break down/modify host lipid rafts (97), presumably to reduce inflammation.

Shigella infection is accompanied by an increased abundance of rafts in host cells and by insertion into the host lipid rafts of invasin IpaB, required for the binding and invasion of the bacteria (22). *E. coli* K1, after binding to rafts, induces translocation of its binding receptor, Ecgp96, and internalization cofactor, TLR2, into rafts, at the same time inhibiting shifting into rafts of TLR4 (98). Together, these effects allow the pathogen to use the phagocytosis pathway while reducing the inflammatory response. *Pseudomonas aeruginosa*, upon contact with epithelial cells, induces sphingomyelinase, which converts host lipid rafts into larger structures required for internalizing the bacteria; preventing

modification of the rafts also prevents bacterial entry and host cell apoptosis (99). However, raft modification is also a part of host cell's negative regulation of cytokine response to the infection by *P. aeruginosa*, and disruption of lipid rafts leads to an uncontrolled release of IL-1β and sepsis (99), presumably in response to limited but unopposed infection occurring under these suboptimal conditions. This example shows the sometimes overlapping activities of lipid rafts in pathogen offense and cellular defense mechanisms.

ASSEMBLY

Lipid rafts are a platform for assembly of many viruses. The benefit of this preference is the possibility to achieve a high concentration of viral proteins in a confined space, which is a necessary requirement for virion assembly that involves protein multimerization. Again, HIV is among the best studied examples of viruses assembling at lipid rafts. The HIV protein Gag drives virion assembly and is targeted to lipid rafts by N-terminal myristate and a highly basic region at its N terminus (100). Assembly is associated with multimerization of Gag, which coalesces the rafts into bigger structures that can accommodate the size of assembled virions (100–150 nm) (101, 102). Consistent with a role of lipid rafts as a platform to concentrate viral proteins for assembly, assembling Gag molecules largely originate from cytoplasm (103) rather than from the membrane pool through lateral diffusion. Importantly, the envelope proteins of HIV are also segregated into the lipid rafts during virion assembly, and this targeting appears to be independent of the Gag-Env interaction (104, 105). Association of HIV Env with lipid rafts is driven by a palmitoylation signal(s) (106, 107) and lentivirus lytic peptide motifs (108) located in the cytoplasmic tail of gp41.

EXIT

While some pathogens, such as cytolytic viruses, release themselves by killing the host cell and breaking out, others avoid such drastic measures using cell exocytosis machinery, which often localizes and functions in lipid rafts.

Prions, due to their very limited choice of tools, fully rely on host machinery to be released from lipid rafts in EVs, ensuring not only targeted and rapid transmission of the pathogen within the host but also safe passage between the hosts and within the newly infected host (109).

Viruses use different mechanisms of budding, but generally they involve viral proteins interacting with cellular proteins that are part of the host endosomal sorting complex required for transport (ESCRT) machinery to release the virus in a manner similar to exocytosis. ESCRT factors are involved in membrane fission and release of vesicles into endosomal multivesicular bodies (110, 111), a process similar to virus budding from the host cell. The ESCRT pathway is not known to be lipid raft-specific, but a variety of ESCRT proteins were identified in lipid rafts (112). If ESCRT proteins are enriched in lipid rafts, it would facilitate their recruitment to assembling virions, providing an additional basis for selecting these membrane domains as assembly sites. Below, we describe the budding process for HIV-1, but a similar mechanism with certain modifications governs budding of other retroviruses (113).

Analysis of the lipid and protein composition of the HIV membrane strongly suggests that the virus buds out from the host cell lipid rafts using them to build its membrane, which is enriched in sphingomyelin, cholesterol, and raft-specific proteins (114–116). However, such classical markers of lipid rafts as flotillin-1 or raft-associated proteins CD4 and CD14 are not present or are underrepresented in pure HIV-1 preparations (115–117), suggesting that HIV budding involves a specialized raft-clustering process. HIV assembly is terminated and budding initiated by recruitment of two early-acting ESCRT factors, TSG101 and ALIX, to two "late domain" motifs in the p6 region of the HIV-1 Gag precursor protein (118, 119). The "PTAP" late domain that binds TSG101, the subunit of the heterotetrameric ESCRT-I complex (120–122), mimics a cellular ESCRT-I recruiting motif found in proteins in endosomal membranes (123). The "YPXL" domain that binds the ESCRT factor ALIX is mimicking a motif used by cellular ALIX ligands in fungi (124), but nonviral YPXL-containing binding partners for mammalian ALIX remain to be characterized. TSG101 and ALIX then recruit ESCRT-III and regulating ATPase vacuolar protein sorting-associated protein 4 (Vps4), which in turn mediate membrane fission [reviewed in (125)]. Fission may be facilitated at the raft domain boundary by outward bending of raft clusters (126), again underscoring the advantages of lipid rafts as virus assembly and budding platforms.

While the ESCRT-dependent budding mechanism has been adopted by many enveloped viruses, including Ebola virus, rabies, herpes simplex type 1, and hepatitis B (127), some use alternative budding pathways. IAV assembles at and buds from lipid rafts; similar to HIV, virus assembly causes coalescence of the rafts induced by a transmembrane domain of HA (128). However, in contrast to HIV, influenza budding is ESCRT- and Vsp4-independent (129). In fact, influenza virus relies on its own protein, M2, to accomplish bud neck formation and bud neck scission. M2 is a non-raft protein but is brought to the rafts by protein-protein interaction with M1 protein, which in turn is brought to the budding assemblage by HA (130). M2 accumulates at the boundary between the raft-like lipid microdomains and the adjacent plasma membrane and utilizes a line tension in this region to promote bud neck constriction and thinning, ultimately resulting in bud scission (131). The line tension generated on the raft/non-raft boundary is a possible explanation why influenza virus, HIV-1, and many other viruses utilize lipid rafts as their budding platform (132).

Intracellular bacteria also use rafts to get out of the host cell. *Streptococcus* and *Clostridium* species use cytolysins to disrupt membranes to break out, first from the phagosome and then from the cell. Properties of cytolysins vary, but many of them are dependent on asymmetric distribution of cholesterol in the membrane and/or bind to cholesterol or GPI-anchored proteins; it is most likely they bind to

and act in the lipid rafts (133–135). Cytolysin perfringolysin O was even used to map rafts using super-resolution microscopy (136).

LIPID RAFTS AND METABOLIC COMORBIDITIES OF INFECTIONS

Many pathogens secrete EVs, which contain a variety of microbial products, into the bloodstream (137). Some of these products target host lipid rafts, and not just in the cells a pathogen is capable of infecting, but systemically, affecting many cell types in the infected host. Presumably, this activity serves to inactivate host immune defenses and to create a metabolic milieu favoring the infection. Intracellular microbes do this too, affecting the host organism systemically "by mail," when infected cells release microbial products in cell-derived exosomes or other forms of EVs. This action, however, is indiscriminate, triggering collateral changes that do not benefit the pathogen in an obvious way. Cells infected with HIV release EVs containing viral protein Nef (95, 138–140). Nef reduces host protein ABCA1 increasing the abundance of lipid rafts. The likely benefit for the virus is in increasing the abundance of rafts on potential HIV target cells elevating the chances of such cells becoming infected. However, the benefits of increasing the abundance of lipid rafts in cells that cannot be infected with HIV because they do not have HIV receptors are less obvious. For some cells, there may be an indirect benefit for the virus: for example, reduction of ABCA1 in hepatocytes results in hypoalphalipoproteinemia (141), and a low level of high density lipoprotein is associated with accumulation of cellular cholesterol and increased abundance of lipid rafts in monocytes and macrophages, cells that can be infected with HIV. However, it is unclear how increasing raft abundance in neurons or pancreatic cells would benefit the virus while triggering or potentiating such comorbidities as cognitive impairment and diabetes. It can only be assumed that these and many other metabolic comorbidities of HIV infection are collateral damage, a consequence of HIV not being able to target Nef where it is really needed and affecting all cells instead. A similar example is *P. falciparum*. Erythrocytes infected with *P. falciparum* secrete EVs containing miRNAs, which trigger inflammation and endothelial dysfunction (142); the benefits of that for the microbe are unclear.

CONCLUSIONS

Mosaic structure and the presence of lipid rafts are the fundamental elements of the organization of the cellular plasma membrane that are essential for its key functions, to separate and to unite. Evolution of pathogens is tightly linked with the evolution of the host: evolving pathogens adapt to evolving hosts. It is not surprising that one outcome of evolutionary pressure is that many pathogenic microbes, especially the intracellular parasites, take full advantage of the properties and functions of the lipid rafts, hijacking them and exploiting them for their own survival.

Host lipid rafts are utilized by many pathogens at almost every stage of their life cycle to assist in pathogen replication and dissemination, to neutralize host immune defenses, and to create a metabolic environment favoring the infection. The widespread use of rafts by a variety of pathogens opens possibilities for creating "raft-modifying" therapeutic approaches potentially targeting a broad spectrum of infectious agents. Given the physiological importance of the rafts, such a therapeutic agent would be required to provide a measured and controlled modification of the lipid rafts, not an easy task, but examples of such fine-tuned modulation have been described and will be discussed in another article in this series. Furthermore, understanding the mechanisms and consequences of raft modifications by the pathogens often reveals novel physiological pathways and mechanisms relying on the rafts. In this context, pathogens may be viewed not only as a foe worth killing but also as a creative tool, similar to genetic mutations, to be used to generate important knowledge on issues much broader than the pathogenesis of infectious diseases.▪

REFERENCES

1. Simons, K., and M. J. Gerl. 2010. Revitalizing membrane rafts: new tools and insights. *Nat. Rev. Mol. Cell Biol.* **11:** 688–699.
2. Pike, L. J. 2004. Lipid rafts: heterogeneity on the high seas. *Biochem. J.* **378:** 281–292.
3. Bagam, P., D. P. Singh, M. E. Inda, and S. Batra. 2017. Unraveling the role of membrane microdomains during microbial infections. *Cell Biol. Toxicol.* **33:** 429–455.
4. Toledo, A., and J. L. Benach. 2015. Hijacking and use of host lipids by intracellular pathogens. *Microbiol. Spectr.* **3:** doi:10.1128/microbiolspec.VMBF-0001-2014.
5. Sviridov, D., and M. Bukrinsky. 2014. Interaction of pathogens with host cholesterol metabolism. *Curr. Opin. Lipidol.* **25:** 333–338.
6. Millán, J., J. Cerny, V. Horejsi, and M. A. Alonso. 1999. CD4 segregates into specific detergent-resistant T-cell membrane microdomains. *Tissue Antigens.* **53:** 33–40.
7. Popik, W., T. M. Alce, and W. C. Au. 2002. Human immunodeficiency virus type 1 uses lipid raft-colocalized CD4 and chemokine receptors for productive entry into CD4(+) T cells. *J. Virol.* **76:** 4709–4722.
8. Kozak, S. L., J. M. Heard, and D. Kabat. 2002. Segregation of CD4 and CXCR4 into distinct lipid microdomains in T lymphocytes suggests a mechanism for membrane destabilization by human immunodeficiency virus. *J. Virol.* **76:** 1802–1815.
9. Kamiyama, H., H. Yoshii, Y. Tanaka, H. Sato, N. Yamamoto, and Y. Kubo. 2009. Raft localization of CXCR4 is primarily required for X4-tropic human immunodeficiency virus type 1 infection. *Virology.* **386:** 23–31.
10. Chazal, N., and D. Gerlier. 2003. Virus entry, assembly, budding, and membrane rafts. *Microbiol. Mol. Biol. Rev.* **67:** 226–237.
11. Lim, K. I., and J. Yin. 2006. Dynamic tradeoffs in the raft-mediated entry of human immunodeficiency virus type 1 into cells. *Biotechnol. Bioeng.* **93:** 246–257.
12. Alfano, M., H. Schmidtmayerova, C. A. Amella, T. Pushkarsky, and M. Bukrinsky. 1999. The B-oligomer of pertussis toxin deactivates CC chemokine receptor 5 and blocks entry of M-tropic HIV-1 strains. *J. Exp. Med.* **190:** 597–605.
13. Groux-Degroote, S., Y. Guerardel, and P. Delannoy. 2017. Gangliosides: structures, biosynthesis, analysis, and roles in cancer. *ChemBioChem.* **18:** 1146–1154.
14. Fujioka, Y., S. Nishide, T. Ose, T. Suzuki, I. Kato, H. Fukuhara, M. Fujioka, K. Horiuchi, A. O. Satoh, P. Nepal, et al. 2018. A sialylated voltage-dependent Ca2+ channel binds hemagglutinin and mediates influenza A virus entry into mammalian cells. *Cell Host Microbe.* **23:** 809–818.e5.
15. Ho, J. W., O. Hershkovitz, M. Peiris, A. Zilka, A. Bar-Ilan, B. Nal, K. Chu, M. Kudelko, Y. W. Kam, H. Achdout, et al. 2008. H5-type

influenza virus hemagglutinin is functionally recognized by the natural killer-activating receptor NKp44. *J. Virol.* **82:** 2028–2032.

16. Eierhoff, T., E. R. Hrincius, U. Rescher, S. Ludwig, and C. Ehrhardt. 2010. The epidermal growth factor receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. *PLoS Pathog.* **6:** e1001099.

17. Mandelboim, O., N. Lieberman, M. Lev, L. Paul, T. I. Arnon, Y. Bushkin, D. M. Davis, J. L. Strominger, J. W. Yewdell, and A. Porgador. 2001. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature.* **409:** 1055–1060.

18. Du, R., Q. Cui, and L. Rong. 2019. Competitive cooperation of hemagglutinin and neuraminidase during influenza A virus entry. *Viruses.* **11:** E458.

19. Lee, J. K., G. A. Enciso, D. Boassa, C. N. Chander, T. H. Lou, S. S. Pairawan, M. C. Guo, F. Y. M. Wan, M. H. Ellisman, C. Sutterlin, et al. 2018. Replication-dependent size reduction precedes differentiation in Chlamydia trachomatis. *Nat. Commun.* **9:** 45.

20. Stuart, E. S., W. C. Webley, and L. C. Norkin. 2003. Lipid rafts, caveolae, caveolin-1, and entry by Chlamydiae into host cells. *Exp. Cell Res.* **287:** 67–78.

21. Kim, J. H., S. Jiang, C. A. Elwell, and J. N. Engel. 2011. Chlamydia trachomatis co-opts the FGF2 signaling pathway to enhance infection. *PLoS Pathog.* **7:** e1002285.

22. Lafont, F., G. Tran Van Nhieu, K. Hanada, P. Sansonetti, and F. G. van der Goot. 2002. Initial steps of Shigella infection depend on the cholesterol/sphingolipid raft-mediated CD44-IpaB interaction. *EMBO J.* **21:** 4449–4457.

23. Yam, X. Y., C. Birago, F. Fratini, F. Di Girolamo, C. Raggi, M. Sargiacomo, A. Bachi, L. Berry, G. Fall, C. Curra, et al. 2013. Proteomic analysis of detergent-resistant membrane microdomains in trophozoite blood stage of the human malaria parasite Plasmodium falciparum. *Mol. Cell. Proteomics.* **12:** 3948–3961.

24. Weiss, G. E., P. R. Gilson, T. Taechalertpaisarn, W. H. Tham, N. W. de Jong, K. L. Harvey, F. J. Fowkes, P. N. Barlow, J. C. Rayner, G. J. Wright, et al. 2015. Revealing the sequence and resulting cellular morphology of receptor-ligand interactions during Plasmodium falciparum invasion of erythrocytes. *PLoS Pathog.* **11:** e1004670.

25. Prusiner, S. B. 1989. Scrapie prions. *Annu. Rev. Microbiol.* **43:** 345–374.

26. Taylor, D. R., and N. M. Hooper. 2006. The prion protein and lipid rafts. *Mol. Membr. Biol.* **23:** 89–99.

27. Rouvinski, A., S. Karniely, M. Kounin, S. Moussa, M. D. Goldberg, G. Warburg, R. Lyakhovetsky, D. Papy-Garcia, J. Kutzsche, C. Korth, et al. 2014. Live imaging of prions reveals nascent PrPSc in cell-surface, raft-associated amyloid strings and webs. *J. Cell Biol.* **204:** 423–441.

28. Cui, H. L., B. Guo, B. Scicluna, B. M. Coleman, V. A. Lawson, L. Ellett, P. J. Meikle, M. Bukrinsky, N. Mukhamedova, D. Sviridov, et al. 2014. Prion infection impairs cholesterol metabolism in neuronal cells. *J. Biol. Chem.* **289:** 789–802.

29. Sarnataro, D., A. Caputo, P. Casanova, C. Puri, S. Paladino, S. S. Tivodar, V. Campana, C. Tacchetti, and C. Zurzolo. 2009. Lipid rafts and clathrin cooperate in the internalization of PrP in epithelial FRT cells. *PLoS One.* **4:** e5829.

30. Cossart, P., and A. Helenius. 2014. Endocytosis of viruses and bacteria. *Cold Spring Harb. Perspect. Biol.* **6:** a016972.

31. Kathuria, R., A. K. Mondal, R. Sharma, S. Bhattacharyya, and K. Chattopadhyay. 2018. Revisiting the role of cholesterol in regulating the pore-formation mechanism of Vibrio cholerae cytolysin, a membrane-damaging beta-barrel pore-forming toxin. *Biochem. J.* **475:** 3039–3055.

32. Rawat, S. S., M. Viard, S. A. Gallo, R. Blumenthal, and A. Puri. 2006. Sphingolipids, cholesterol, and HIV-1: a paradigm in viral fusion. *Glycoconj. J.* **23:** 189–197.

33. Kielian, M., C. Chanel-Vos, and M. Liao. 2010. Alphavirus entry and membrane fusion. *Viruses.* **2:** 796–825.

34. Waarts, B. L., R. Bittman, and J. Wilschut. 2002. Sphingolipid and cholesterol dependence of alphavirus membrane fusion. Lack of correlation with lipid raft formation in target liposomes. *J. Biol. Chem.* **277:** 38141–38147.

35. Tong, J., P. P. Borbat, J. H. Freed, and Y. K. Shin. 2009. A scissors mechanism for stimulation of SNARE-mediated lipid mixing by cholesterol. *Proc. Natl. Acad. Sci. USA.* **106:** 5141–5146.

36. Kwon, B., M. Lee, A. J. Waring, and M. Hong. 2018. Oligomeric structure and three-dimensional fold of the HIV gp41 membrane-proximal external region and transmembrane domain in phospholipid bilayers. *J. Am. Chem. Soc.* **140:** 8246–8259.

37. Kollmitzer, B., P. Heftberger, M. Rappolt, and G. Pabst. 2013. Monolayer spontaneous curvature of raft-forming membrane lipids. *Soft Matter.* **9:** 10877–10884.

38. Biswas, S., S. R. Yin, P. S. Blank, and J. Zimmerberg. 2008. Cholesterol promotes hemifusion and pore widening in membrane fusion induced by influenza hemagglutinin. *J. Gen. Physiol.* **131:** 503–513.

39. Yang, S. T., V. Kiessling, J. A. Simmons, J. M. White, and L. K. Tamm. 2015. HIV gp41-mediated membrane fusion occurs at edges of cholesterol-rich lipid domains. *Nat. Chem. Biol.* **11:** 424–431.

40. Yang, S. T., V. Kiessling, and L. K. Tamm. 2016. Line tension at lipid phase boundaries as driving force for HIV fusion peptide-mediated fusion. *Nat. Commun.* **7:** 11401.

41. Gaus, K., E. Chklovskaia, B. Fazekas de St Groth, W. Jessup, and T. Harder. 2005. Condensation of the plasma membrane at the site of T lymphocyte activation. *J. Cell Biol.* **171:** 121–131.

42. Biancotto, A., S. J. Iglehart, C. Vanpouille, C. E. Condack, A. Lisco, E. Ruecker, I. Hirsch, L. B. Margolis, and J. C. Grivel. 2008. HIV-1 induced activation of CD4+ T cells creates new targets for HIV-1 infection in human lymphoid tissue ex vivo. *Blood.* **111:** 699–704.

43. Chen, B. 2019. Molecular mechanism of HIV-1 entry. *Trends Microbiol.* **27:** 878–891.

44. Dumas, F., P. Preira, and L. Salome. 2014. Membrane organization of virus and target cell plays a role in HIV entry. *Biochimie.* **107 Pt A:** 22–27.

45. Stein, B. S., S. D. Gowda, J. D. Lifson, R. C. Penhallow, K. G. Bensch, and E. G. Engleman. 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. *Cell.* **49:** 659–668.

46. Goto, T., S. Harada, N. Yamamoto, and M. Nakai. 1988. Entry of human immunodeficiency virus (HIV) into MT-2, human T cell leukemia virus carrier cell line. *Arch. Virol.* **102:** 29–38.

47. Schaeffer, E., V. B. Soros, and W. C. Greene. 2004. Compensatory link between fusion and endocytosis of human immunodeficiency virus type 1 in human CD4 T lymphocytes. *J. Virol.* **78:** 1375–1383.

48. Miyauchi, K., Y. Kim, O. Latinovic, V. Morozov, and G. B. Melikyan. 2009. HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell.* **137:** 433–444.

49. Herold, N., M. Anders-Osswein, B. Glass, M. Eckhardt, B. Muller, and H. G. Krausslich. 2014. HIV-1 entry in SupT1-R5, CEM-ss, and primary CD4+ T cells occurs at the plasma membrane and does not require endocytosis. *J. Virol.* **88:** 13956–13970.

50. Foster, T. L., H. Wilson, S. S. Iyer, K. Coss, K. Doores, S. Smith, P. Kellam, A. Finzi, P. Borrow, B. H. Hahn, et al. 2016. Resistance of transmitted founder HIV-1 to IFITM-mediated restriction. *Cell Host Microbe.* **20:** 429–442.

51. Herold, N. 2019. Overexpression of the interferon-inducible isoform 4 of NCOA7 dissects the entry route of enveloped viruses and demonstrates that HIV enters cells via fusion at the plasma membrane. *Viruses.* **11:** E121.

52. Norkin, L. C., S. A. Wolfrom, and E. S. Stuart. 2001. Association of caveolin with Chlamydia trachomatis inclusions at early and late stages of infection. *Exp. Cell Res.* **266:** 229–238.

53. Luo, M. 2012. Influenza virus entry. *Adv. Exp. Med. Biol.* **726:** 201–221.

54. Verma, D. K., D. Gupta, and S. K. Lal. 2018. Host lipid rafts play a major role in binding and endocytosis of influenza A virus. *Viruses.* **10:** E650.

55. Gerlach, T., L. Hensen, T. Matrosovich, J. Bergmann, M. Winkler, C. Peteranderl, H. D. Klenk, F. Weber, S. Herold, S. Pohlmann, et al. 2017. pH optimum of hemagglutinin-mediated membrane fusion determines sensitivity of influenza A viruses to the interferon-induced antiviral state and IFITMs. *J. Virol.* **91:** e00246-17.

56. Fontana, J., and A. C. Steven. 2015. Influenza virus-mediated membrane fusion: structural insights from electron microscopy. *Arch. Biochem. Biophys.* **581:** 86–97.

57. Helenius, A. 1992. Unpacking the incoming influenza virus. *Cell.* **69:** 577–578.

58. Veit, M., and B. Thaa. 2011. Association of influenza virus proteins with membrane rafts. *Adv. Virol.* **2011:** 370606.

59. Thaa, B., I. Levental, A. Herrmann, and M. Veit. 2011. Intrinsic membrane association of the cytoplasmic tail of influenza virus M2 protein and lateral membrane sorting regulated by cholesterol binding and palmitoylation. *Biochem. J.* **437:** 389–397.

60. Bavari, S., C. M. Bosio, E. Wiegand, G. Ruthel, A. B. Will, T. W. Geisbert, M. Hevey, C. Schmaljohn, A. Schmaljohn, and M. J.

Aman. 2002. Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses. *J. Exp. Med.* **195:** 593–602.

61. Aman, M. J., C. M. Bosio, R. G. Panchal, J. C. Burnett, A. Schmaljohn, and S. Bavari. 2003. Molecular mechanisms of filovirus cellular trafficking. *Microbes Infect.* **5:** 639–649.

62. Chan, S. Y., R. F. Speck, M. C. Ma, and M. A. Goldsmith. 2000. Distinct mechanisms of entry by envelope glycoproteins of Marburg and Ebola (Zaire) viruses. *J. Virol.* **74:** 4933–4937.

63. Kondratowicz, A. S., N. J. Lennemann, P. L. Sinn, R. A. Davey, C. L. Hunt, S. Moller-Tank, D. K. Meyerholz, P. Rennert, R. F. Mullins, M. Brindley, et al. 2011. T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. *Proc. Natl. Acad. Sci. USA.* **108:** 8426–8431.

64. Rhein, B. A., R. B. Brouillette, G. A. Schaack, J. A. Chiorini, and W. Maury. 2016. Characterization of human and murine T-cell immunoglobulin mucin domain 4 (TIM-4) IgV domain residues critical for Ebola virus entry. *J. Virol.* **90:** 6097–6111.

65. Loh, L. N., E. M. C. McCarthy, P. Narang, N. A. Khan, and T. H. Ward. 2017. Escherichia coli K1 utilizes host macropinocytic pathways for invasion of brain microvascular endothelial cells. *Traffic.* **18:** 733–746.

66. Sukumaran, S. K., H. Shimada, and N. V. Prasadarao. 2003. Entry and intracellular replication of Escherichia coli K1 in macrophages require expression of outer membrane protein A. *Infect. Immun.* **71:** 5951–5961.

67. Diesselberg, C., S. Ribes, J. Seele, A. Kaufmann, S. Redlich, S. Bunkowski, U. K. Hanisch, U. Michel, R. Nau, and S. Schutze. 2018. Activin A increases phagocytosis of Escherichia coli K1 by primary murine microglial cells activated by toll-like receptor agonists. *J. Neuroinflammation.* **15:** 175.

68. Yamaguchi, M., Y. Terao, Y. Mori-Yamaguchi, H. Domon, Y. Sakaue, T. Yagi, K. Nishino, A. Yamaguchi, V. Nizet, and S. Kawabata. 2013. Streptococcus pneumoniae invades erythrocytes and utilizes them to evade human innate immunity. *PLoS One.* **8:** e77282.

69. Saito, A., E. Kokubu, S. Inagaki, K. Imamura, D. Kita, R. J. Lamont, and K. Ishihara. 2012. Porphyromonas gingivalis entry into gingival epithelial cells modulated by Fusobacterium nucleatum is dependent on lipid rafts. *Microb. Pathog.* **53:** 234–242.

70. de Turris, V., R. Teloni, P. Chiani, C. Bromuro, S. Mariotti, M. Pardini, R. Nisini, A. Torosantucci, and M. C. Gagliardi. 2015. Candida albicans targets a lipid raft/dectin-1 platform to enter human monocytes and induce antigen specific T cell responses. *PLoS One.* **10:** e0142531.

71. Guimarães, A. J., M. D. de Cerqueira, D. Zamith-Miranda, P. H. Lopez, M. L. Rodrigues, B. Pontes, N. B. Viana, C. M. DeLeon-Rodriguez, D. C. P. Rossi, A. Casadevall, et al. 2019. Host membrane glycosphingolipids and lipid microdomains facilitate Histoplasma capsulatum internalisation by macrophages. *Cell. Microbiol.* **21:** e12976.

72. Rönnebäumer, K., U. Gross, and W. Bohne. 2008. The nascent parasitophorous vacuole membrane of Encephalitozoon cuniculi is formed by host cell lipids and contains pores which allow nutrient uptake. *Eukaryot. Cell.* **7:** 1001–1008.

73. Pedro, K. D., A. J. Henderson, and L. M. Agosto. 2019. Mechanisms of HIV-1 cell-to-cell transmission and the establishment of the latent reservoir. *Virus Res.* **265:** 115–121.

74. Noriega, V. M., T. J. Gardner, V. Redmann, G. Bongers, S. A. Lira, and D. Tortorella. 2014. Human cytomegalovirus US28 facilitates cell-to-cell viral dissemination. *Viruses.* **6:** 1202–1218.

75. Omsland, M., C. Pise-Masison, D. Fujikawa, V. Galli, C. Fenizia, R. W. Parks, B. T. Gjertsen, G. Franchini, and V. Andresen. 2018. Inhibition of tunneling nanotube (TNT) formation and human T-cell leukemia virus type 1 (HTLV-1) transmission by cytarabine. *Sci. Rep.* **8:** 11118.

76. Bayliss, R. J., and V. Piguet. 2018. Masters of manipulation: viral modulation of the immunological synapse. *Cell. Microbiol.* **20:** e12944.

77. Sattentau, Q. 2008. Avoiding the void: cell-to-cell spread of human viruses. *Nat. Rev. Microbiol.* **6:** 815–826.

78. Real, F., A. Sennepin, Y. Ganor, A. Schmitt, and M. Bomsel. 2018. Live imaging of HIV-1 transfer across T cell virological synapse to epithelial cells that promotes stromal macrophage infection. *Cell Reports.* **23:** 1794–1805.

79. Jolly, C., and Q. J. Sattentau. 2005. Human immunodeficiency virus type 1 virological synapse formation in T cells requires lipid raft integrity. *J. Virol.* **79:** 12088–12094.

80. Rai, A., D. Pathak, S. Thakur, S. Singh, A. K. Dubey, and R. Mallik. 2016. Dynein clusters into lipid microdomains on phagosomes to drive rapid transport toward lysosomes. *Cell.* **164:** 722–734.

81. Kim, J. Y., L. Wang, J. Lee, and J. J. Ou. 2017. Hepatitis C virus induces the localization of lipid rafts to autophagosomes for its RNA replication. *J. Virol.* **91:** e00541-17.

82. Lin, M., and Y. Rikihisa. 2003. Obligatory intracellular parasitism by Ehrlichia chaffeensis and Anaplasma phagocytophilum involves caveolae and glycosylphosphatidylinositol-anchored proteins. *Cell. Microbiol.* **5:** 809–820.

83. Coppens, I. 2017. How Toxoplasma and malaria parasites defy first, then exploit host autophagic and endocytic pathways for growth. *Curr. Opin. Microbiol.* **40:** 32–39.

84. Carabeo, R. A., D. J. Mead, and T. Hackstadt. 2003. Golgi-dependent transport of cholesterol to the Chlamydia trachomatis inclusion. *Proc. Natl. Acad. Sci. USA.* **100:** 6771–6776.

85. Schraw, W., Y. Li, M. S. McClain, F. G. van der Goot, and T. L. Cover. 2002. Association of Helicobacter pylori vacuolating toxin (VacA) with lipid rafts. *J. Biol. Chem.* **277:** 34642–34650.

86. Morey, P., L. Pfannkuch, E. Pang, F. Boccellato, M. Sigal, A. Imai-Matsushima, V. Dyer, M. Koch, H. J. Mollenkopf, P. Schlaermann, et al. 2018. Helicobacter pylori depletes cholesterol in gastric glands to prevent interferon gamma signaling and escape the inflammatory response. *Gastroenterology.* **154:** 1391–1404.e9.

87. Wunder, C., Y. Churin, F. Winau, D. Warnecke, M. Vieth, B. Lindner, U. Zahringer, H-J. Mollenkopf, E. Heinz, and T. F. Meyer. 2006. Cholesterol glucosylation promotes immune evasion by Helicobacter pylori. *Nat. Med.* **12:** 1030–1038.

88. Lai, C. H., J. C. Huang, H. H. Cheng, M. C. Wu, M. Z. Huang, H. Y. Hsu, Y. A. Chen, C. Y. Hsu, Y. J. Pan, Y. T. Chu, et al. 2018. Helicobacter pylori cholesterol glucosylation modulates autophagy for increasing intracellular survival in macrophages. *Cell. Microbiol.* **20:** e12947.

89. Crowley, J. T., A. M. Toledo, T. J. LaRocca, J. L. Coleman, E. London, and J. L. Benach. 2013. Lipid exchange between Borrelia burgdorferi and host cells. *PLoS Pathog.* **9:** e1003109.

90. LaRocca, T. J., P. Pathak, S. Chiantia, A. Toledo, J. R. Silvius, J. L. Benach, and E. London. 2013. Proving lipid rafts exist: membrane domains in the prokaryote Borrelia burgdorferi have the same properties as eukaryotic lipid rafts. *PLoS Pathog.* **9:** e1003353.

91. Cui, H. L., A. Grant, N. Mukhamedova, T. Pushkarsky, L. Jennelle, L. Dubrovsky, K. Gaus, M. L. Fitzgerald, D. Sviridov, and M. Bukrinsky. 2012. HIV-1 Nef mobilizes lipid rafts in macrophages through a pathway that competes with ABCA1-dependent cholesterol efflux. *J. Lipid Res.* **53:** 696–708.

92. Zheng, Y. H., A. Plemenitas, T. Linnemann, O. T. Fackler, and B. M. Peterlin. 2001. Nef increases infectivity of HIV via lipid rafts. *Curr. Biol.* **11:** 875–879.

93. Zheng, Y. H., A. Plemenitas, C. J. Fielding, and B. M. Peterlin. 2003. Nef increases the synthesis of and transports cholesterol to lipid rafts and HIV-1 progeny virions. *Proc. Natl. Acad. Sci. USA.* **100:** 8460–8465.

94. Pan, X., H. M. Baldauf, O. T. Keppler, and O. T. Fackler. 2013. Restrictions to HIV-1 replication in resting CD4+ T lymphocytes. *Cell Res.* **23:** 876–885.

95. Mukhamedova, N., A. Hoang, D. Dragoljevic, L. Dubrovsky, T. Pushkarsky, H. Low, M. Ditiatkovski, Y. Fu, R. Ohkawa, P. J. Meikle, et al. 2019. Exosomes containing HIV protein Nef reorganize lipid rafts potentiating inflammatory response in bystander cells. *PLoS Pathog.* **15:** e1007907.

96. Bomberger, J. M., D. P. Maceachran, B. A. Coutermarsh, S. Ye, G. A. O'Toole, and B. A. Stanton. 2009. Long-distance delivery of bacterial virulence factors by Pseudomonas aeruginosa outer membrane vesicles. *PLoS Pathog.* **5:** e1000382.

97. Low, H., N. Mukhamedova, H. L. Cui, B. P. McSharry, S. Avdic, A. Hoang, M. Ditiatkovski, Y. Liu, Y. Fu, P. J. Meikle, et al. 2016. Cytomegalovirus restructures lipid rafts via a US28/CDC42-mediated pathway, enhancing cholesterol efflux from host cells. *Cell Reports.* **16:** 186–200.

98. Krishnan, S., S. Chen, G. Turcatel, M. Arditi, and N. V. Prasadarao. 2013. Regulation of Toll-like receptor 2 interaction with Ecgp96 controls Escherichia coli K1 invasion of brain endothelial cells. *Cell. Microbiol.* **15:** 63–81.

99. Grassmé, H., V. Jendrossek, A. Riehle, G. von Kürthy, J. Berger, H. Schwarz, M. Weller, R. Kolesnick, and E. Gulbins. 2003. Host defense against Pseudomonas aeruginosa requires ceramide-rich membrane rafts. *Nat. Med.* **9:** 322–330.

100. Ono, A. 2010. Relationships between plasma membrane microdomains and HIV-1 assembly. *Biol. Cell.* **102:** 335–350.

101. Ono, A., and E. O. Freed. 2001. Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc. Natl. Acad. Sci. USA.* **98:** 13925–13930.

102. Lindwasser, O. W., and M. D. Resh. 2001. Multimerization of human immunodeficiency virus type 1 Gag promotes its localization to barges, raft-like membrane microdomains. *J. Virol.* **75:** 7913–7924.

103. Ivanchenko, S., W. J. Godinez, M. Lampe, H. G. Krausslich, R. Eils, K. Rohr, C. Brauchle, B. Muller, and D. C. Lamb. 2009. Dynamics of HIV-1 assembly and release. *PLoS Pathog.* **5:** e1000652.

104. Chan, W. E., H. H. Lin, and S. S. Chen. 2005. Wild-type-like viral replication potential of human immunodeficiency virus type 1 envelope mutants lacking palmitoylation signals. *J. Virol.* **79:** 8374–8387.

105. Leung, K., J. O. Kim, L. Ganesh, J. Kabat, O. Schwartz, and G. J. Nabel. 2008. HIV-1 assembly: viral glycoproteins segregate quantally to lipid rafts that associate individually with HIV-1 capsids and virions. *Cell Host Microbe.* **3:** 285–292.

106. Bhattacharya, J., P. J. Peters, and P. R. Clapham. 2004. Human immunodeficiency virus type 1 envelope glycoproteins that lack cytoplasmic domain cysteines: impact on association with membrane lipid rafts and incorporation onto budding virus particles. *J. Virol.* **78:** 5500–5506.

107. Rousso, I., M. B. Mixon, B. K. Chen, and P. S. Kim. 2000. Palmitoylation of the HIV-1 envelope glycoprotein is critical for viral infectivity. *Proc. Natl. Acad. Sci. USA.* **97:** 13523–13525.

108. Yang, P., L. S. Ai, S. C. Huang, H. F. Li, W. E. Chan, C. W. Chang, C. Y. Ko, and S. S. Chen. 2010. The cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein gp41 harbors lipid raft association determinants. *J. Virol.* **84:** 59–75.

109. Bellingham, S. A., B. B. Guo, B. M. Coleman, and A. F. Hill. 2012. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? *Front. Physiol.* **3:** 124.

110. Hurley, J. H., and P. I. Hanson. 2010. Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nat. Rev. Mol. Cell Biol.* **11:** 556–566.

111. Peel, S., P. Macheboeuf, N. Martinelli, and W. Weissenhorn. 2011. Divergent pathways lead to ESCRT-III-catalyzed membrane fission. *Trends Biochem. Sci.* **36:** 199–210.

112. Dubois, L., K. K. Ronquist, B. Ek, G. Ronquist, and A. Larsson. 2015. Proteomic profiling of detergent resistant membranes (lipid rafts) of prostasomes. *Mol. Cell. Proteomics.* **14:** 3015–3022.

113. Pincetic, A., and J. Leis. 2009. The mechanism of budding of retroviruses from cell membranes. *Adv. Virol.* **2009:** 6239691–6239699.

114. Aloia, R. C., H. Tian, and F. C. Jensen. 1993. Lipid composition and fluidity of the human immunodeficiency virus envelope and host cell plasma membranes. *Proc. Natl. Acad. Sci. USA.* **90:** 5181–5185.

115. Brügger, B., B. Glass, P. Haberkant, I. Leibrecht, F. T. Wieland, and H. G. Krausslich. 2006. The HIV lipidome: a raft with an unusual composition. *Proc. Natl. Acad. Sci. USA.* **103:** 2641–2646.

116. Burnie, J., and C. Guzzo. 2019. The incorporation of host proteins into the external HIV-1 envelope. *Viruses.* **11:** E85.

117. Esser, M. T., D. R. Graham, L. V. Coren, C. M. Trubey, J. W. Bess, Jr., L. O. Arthur, D. E. Ott, and J. D. Lifson. 2001. Differential incorporation of CD45, CD80 (B7-1), CD86 (B7-2), and major histocompatibility complex class I and II molecules into human immunodeficiency virus type 1 virions and microvesicles: implications for viral pathogenesis and immune regulation. *J. Virol.* **75:** 6173–6182.

118. Usami, Y., S. Popov, E. Popova, M. Inoue, W. Weissenhorn, and H. G. Göttlinger. 2009. The ESCRT pathway and HIV-1 budding. *Biochem. Soc. Trans.* **37:** 181–184.

119. Morita, E., and W. I. Sundquist. 2004. Retrovirus budding. *Annu. Rev. Cell Dev. Biol.* **20:** 395–425.

120. Garrus, J. E., U. K. von Schwedler, O. W. Pornillos, S. G. Morham, K. H. Zavitz, H. E. Wang, D. A. Wettstein, K. M. Stray, M. Cote, R. L. Rich, et al. 2001. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell.* **107:** 55–65.

121. Martin-Serrano, J., T. Zang, and P. D. Bieniasz. 2001. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat. Med.* **7:** 1313–1319.

122. VerPlank, L., F. Bouamr, T. J. LaGrassa, B. Agresta, A. Kikonyogo, J. Leis, and C. A. Carter. 2001. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc. Natl. Acad. Sci. USA.* **98:** 7724–7729.

123. Ren, X., and J. H. Hurley. 2011. Proline-rich regions and motifs in trafficking: from ESCRT interaction to viral exploitation. *Traffic.* **12:** 1282–1290.

124. Vincent, O., L. Rainbow, J. Tilburn, H. N. Arst, Jr., and M. A. Penalva. 2003. YPXL/I is a protein interaction motif recognized by aspergillus PalA and its human homologue, AIP1/Alix. *Mol. Cell. Biol.* **23:** 1647–1655.

125. Sundquist, W. I., and H. G. Krausslich. 2012. HIV-1 assembly, budding, and maturation. *Cold Spring Harb. Perspect. Med.* **2:** a006924.

126. Schuck, S., and K. Simons. 2004. Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* **117:** 5955–5964.

127. Bieniasz, P. D. 2006. Late budding domains and host proteins in enveloped virus release. *Virology.* **344:** 55–63.

128. Schmitt, A. P., and R. A. Lamb. 2005. Influenza virus assembly and budding at the viral budozone. *Adv. Virus Res.* **64:** 383–416.

129. Rossman, J. S., X. Jing, G. P. Leser, and R. A. Lamb. 2010. Influenza virus M2 protein mediates ESCRT-independent membrane scission. *Cell.* **142:** 902–913.

130. Nayak, D. P., R. A. Balogun, H. Yamada, Z. H. Zhou, and S. Barman. 2009. Influenza virus morphogenesis and budding. *Virus Res.* **143:** 147–161.

131. Kuzmin, P. I., S. A. Akimov, Y. A. Chizmadzhev, J. Zimmerberg, and F. S. Cohen. 2005. Line tension and interaction energies of membrane rafts calculated from lipid splay and tilt. *Biophys. J.* **88:** 1120–1133.

132. Nayak, D. P., and E. K. Hui. 2004. The role of lipid microdomains in virus biology. *Subcell. Biochem.* **37:** 443–491.

133. Ogasawara, F., F. Kano, M. Murata, Y. Kimura, N. Kioka, and K. Ueda. 2019. Changes in the asymmetric distribution of cholesterol in the plasma membrane influence streptolysin O pore formation. *Sci. Rep.* **9:** 4548.

134. Farrand, A. J., E. M. Hotze, T. K. Sato, K. R. Wade, W. C. Wimley, A. E. Johnson, and R. K. Tweten. 2015. The cholesterol-dependent cytolysin membrane-binding interface discriminates lipid environments of cholesterol to support β-barrel pore insertion. *J. Biol. Chem.* **290:** 17733–17744.

135. Taylor, S. D., M. E. Sanders, N. A. Tullos, S. J. Stray, E. W. Norcross, L. S. McDaniel, and M. E. Marquart. 2013. The cholesterol-dependent cytolysin pneumolysin from Streptococcus pneumoniae binds to lipid raft microdomains in human corneal epithelial cells. *PLoS One.* **8:** e61300.

136. He, C., X. Hu, R. S. Jung, T. A. Weston, N. P. Sandoval, P. Tontonoz, M. R. Kilburn, L. G. Fong, S. G. Young, and H. Jiang. 2017. High-resolution imaging and quantification of plasma membrane cholesterol by NanoSIMS. *Proc. Natl. Acad. Sci. USA.* **114:** 2000–2005.

137. Silverman, J. M., and N. E. Reiner. 2011. Exosomes and other microvesicles in infection biology: organelles with unanticipated phenotypes. *Cell. Microbiol.* **13:** 1–9.

138. McNamara, R. P., L. M. Costantini, T. A. Myers, B. Schouest, N. J. Maness, J. D. Griffith, B. A. Damania, A. G. MacLean, and D. P. Dittmer. 2018. Nef secretion into extracellular vesicles or exosomes is conserved across human and simian immunodeficiency viruses. *MBio.* **9:** e02344-17.

139. Pužar Dominkuš, P., J. Ferdin, A. Plemenitaš, B. M. Peterlin, and M. Lenassi. 2017. Nef is secreted in exosomes from Nef.GFP-expressing and HIV-1-infected human astrocytes. *J. Neurovirol.* **23:** 713–724.

140. Raymond, A. D., P. Diaz, S. Chevelon, M. Agudelo, A. Yndart-Arias, H. Ding, A. Kaushik, R. D. Jayant, R. Nikkhah-Moshaie, U. Roy, et al. 2016. Microglia-derived HIV Nef+ exosome impairment of the blood-brain barrier is treatable by nanomedicine-based delivery of Nef peptides. *J. Neurovirol.* **22:** 129–139.

141. Cui, H. L., M. Ditiatkovski, R. Kesani, Y. V. Bobryshev, Y. Liu, M. Geyer, N. Mukhamedova, M. Bukrinsky, and D. Sviridov. 2014. HIV protein Nef causes dyslipidemia and formation of foam cells in mouse models of atherosclerosis. *FASEB J.* **28:** 2828–2839.

142. Mantel, P. Y., D. Hjelmqvist, M. Walch, S. Kharoubi-Hess, S. Nilsson, D. Ravel, M. Ribeiro, C. Gruring, S. Ma, P. Padmanabhan, et al. 2016. Infected erythrocyte-derived extracellular vesicles alter vascular function via regulatory Ago2-miRNA complexes in malaria. *Nat. Commun.* **7:** 12727.