Published in final edited form as:
Adv Exp Med Biol. 2012 ; 729: 65–82. doi:10.1007/978-1-4614-1222-9_5.

RECENT DEVELOPMENTS IN THE INTERACTIONS BETWEEN CAVEOLIN AND PATHOGENS

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Abstract

The role of caveolin and caveolae in the pathogenesis of infection has only recently been appreciated. In this chapter, we have highlighted some important new data on the role of caveolin in infections due to bacteria, viruses and fungi but with particular emphasis on the protozoan parasites *Leishmania spp., Trypanosoma cruzi* and *Toxoplasma gondii.* This is a continuing area of research and the final chapter has not been written on this topic.

INTRODUCTION

The first steps in the initiation of an infection are the attachment and entry of a pathogen into a host cell. It has long been assumed that an understanding of these initial events may result in new methods for control and treatment of infections. A role for caveolae and caveolin

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proteins in these processes has only recently been investigated. In this chapter, it is not our intent to review all of microbiology and describe how each and every micro-organism interacts with caveolae and caveolin proteins, but rather to focus our attention on some recent important new developments. Pathogens enter mammalian cells to escape the immune system of the host and/or as part of their requirement to maintain a replicative cycle. In the past several years, this topic has been reviewed by others.[1–5] These articles highlighted the role of caveolae and caveolin proteins, such as caveolin-1 (Cav-1) and lipid rafts in the entry of diverse pathogens into the host cell. As discussed in detail in other chapters in this book, Cav-1 is a critical structural protein in the formation of the flask shaped caveolae lining the plasma membrane. In addition, the role of caveolins in the pathogenesis of infection may be related to an effect on components of the immune system such as lymphocytes and macrophages.[6,7]

VIRUSES

Caveolae are enriched in cholesterol and glycolipids, such as the glycosphingolipid GM1, glycosylphosphatidylinositol-anchored proteins and caveolin. Caveolae–mediated viral entry into human cell lines has been described and viruses that enter cells via caveolae apparently act as signaling ligands, triggering signal transduction events and actin rearrangement in the host cell, resulting in pathogen uptake (Table 1).[8,9]

The mechanisms by which a virus gains entry via caveolae are still not completely understood. The SV40 virus may bind to the MHC Class I complex, which recruits Cav-1 to the site of viral attachment from preformed caveolae and the associated lipid rafts are then formed around the virus. Alternatively, the SV40 virus bound to MHC may be associated with preformed caveolae. Regardless of the precise mechanism, SV40 containing caveolae pinch off from the plasma membrane and are transported to the endoplasmic reticulum via a caveosome.[8] Polyoma virus, Echovirus, Respiratory Syncitial Virus (RSV) and the filoviruses (Ebola and Marburg viruses) are additional examples of viruses that are associated with Cav-1. The effects of lipid raft disrupting agents on Ebola infection indicate that membrane lipid rafts are important in the entry of filoviruses.[1]

The HIV-1 receptors are associated with lipid rafts in T-cells and the disruption of the integrity of these lipid rafts likely inhibits HIV infection. The blood brain barrier (BBB) integrity is maintained by tight junctions,[10,11] and an intact BBB is crucial for preventing the trafficking of HIV into the brain. Early in the course of HIV infection, virus crosses the BBB via HIV-1 infected monocytes in macrophages and microglial cells in the brain. During the subsequent inflammatory response, leukocytes enter into the central nervous system through breaches in the BBB. Weiss et al [12] demonstrated that HIV-1-Tat protein is a powerful pro-inflammatory agent that causes transendothelial cell migration of monocytes. Recently, Zhong et al[13] demonstrated that Tat-mediated activation of Ras signaling is regulated by Cav-1 in brain endothelial cells and that inhibition of Cav-1 and Ras signaling attenuates Tat-induced disruption of the tight junction proteins.

Papillomaviruses (PVs) infect the mucosal and cutaneous stratified squamous epithelia. These infections are associated with both benign and malignant neoplasias and the human papillomaviruses (HPVs) cause virtually all cases of cervical cancer.[14] The 8 kb, circular viral genome is encapsulated by a complex of L1 (major) and L2 (minor) structural proteins.[15] Upon binding to heparan sulfate proteoglycans, the PV capsid undergoes a series of conformational changes resulting in the N-terminus of L2 becoming sensitive to cleavage by furin.[16] Arecent study suggested that these conformational changes may occur on the extracellular matrix prior to transfer to the cell.[17] Earlier work employed chemical inhibitors and/or microscopic localization to suggest that HPV16, HPV33, HPV58 and Bovine PV1

(BPV1) use a clathrin-dependent pathway, whereas similar studies suggest the use of a caveolae-dependent pathway for HPV31.[18–22] However, more recent work suggests that HPV31 may also use a clathrin-dependent pathway.[20] Other authors have suggested that PVs can enter cells independent of either pathway.[23]

The lack of in vitro culture methods for the production of infectious virus has limited the study of PV entry. Most studies have used viral-like particles (VLPs) produced in insect cells or pseudovirions in which the L1/L2 capsid carries a reporter gene. It is possible that the use of these laboratory-made particles and nontarget cell lines could explain the observed differences between entry mechanisms. For example, it has been demonstrated that HPV16 enters dendritic cells and Langerhans cells via distinct pathways.[24]

The observations that a simian virus, JC virus (a polyoma virus), enters cells via clathrin-mediated endocytosis before being shuttled to caveolae-derived vesicles[25] led to the identification of a similar pathway for HPV16 and BPV1.[26,27] Based on recent studies, HPV16 virions first colocalize with markers of early endosomes; then, beginning at around 20 minutes post-entry, increasing amounts of the virons colocalize with Cav-1. Four hours post-entry the HPV16 virions are present in the endoplasmic reticulum. These observations strongly suggest that HPV16 and BPV1 enter the host cell via a clathrin-dependent pathway, after which they get shuttled from endosomes to caveolae. Conversely, HPV31 enters cells through the caveolar pathway followed by Rab5-dependent shuttling towards endosomes.[28] Since Rab5 controls the transport from endosomes to caveosomes and vice-versa, it is tempting to speculate that both HPV16 and HPV31 could shuttle back and forth between the caveolar and endosomal pathways following entry. This hypothesis is supported by the ultrastructural observation that both HPV16 and HPV31 end up in similar looking vesicles.[18] However, studies in which cells have been co-infected with HPV16 and HPV31 have not shown colocalization of both viral types.[23] It is possible that the differences in entry half-time (4 hours for HPV16 vs 14 hours for HPV31) could explain these observations. Thus, it appears that HPV31 has evolved to (predominantly) enter cells through a uniquely different pathway from the other tested PV types. This is surprising since HPV16 and HPV31 are evolutionary more closely related to each other than HPV16 is to HPV58 or BPV1 (HPV16 and HPV31 share approximately 83% amino acid similarity (PAM250 matrix) across the L1 structural protein). One hypothesis is that these two highly related viruses evolved to use different entry mechanisms to avoid competition. However, the much higher prevalence of HPV16 may suggest otherwise. One key observation is that it appears that the HPV16-E5 protein up-regulates Cav-1 at the plasma-membrane of cervical cells. Taken together, these findings suggest that caveolin proteins might play a role in PV entry, but it will be necessary to sort out discrepant results and intertype differences in order to adequately assess their role.

BACTERIA

There is limited information regarding the role of caveolins in bacterial infection. However, early studies suggested that caveolae may be an important alternative pathway for endocytosis of bacteria.[1,2,4,5] Chemical agents have been used to study the function of caveolae including nystatin, filipin and methyl-β-cyclodextrin (MβCD). These agents disrupt the cholesterol enriched lipid rafts. Many pathogens require lipid rafts for entry and colocalize with markers of caveolae to invade host cells. However, only a small number of bacterial pathogens have actually been shown to require caveolin expression for host cell entry.

Escherichia coli is an important cause of human infection including those of the urinary and gastrointestinal tracts. Studies involving *E. coli* invasion into mast and bladder epithelial

cells have revealed that caveolae-dependent endocytosis is a mechanism for bacteria to invade both phagocytic and nonphagocytic cells. Importantly, intracellular bacteria can colocalize with Cav-1 and compounds that cause disruption of caveolae by removing membrane cholesterol inhibited bacterial invasion. *E. coli* uptake and invasion is dependent on the organization of lipid rafts and Cav-1 expression.[29] Furthermore, *Campylobacter jejuni,* is an important cause of diarrhea world-wide whose invasion of intestinal epithelial cells is dependent on Cav-1 expression.[30]

In an intraperitoneal model of sepsis using lipopolysaccharide injection, Cav-1 null mice were observed to be resistant to lung injury and their mortality was reduced due to a reduction in inflammation.[31] However, in a Cav-1 null mouse model *of Salmonella typhimirium,* an important cause of human diarrhea and systemic illness, Medina etal[32] found that higher levels of proinflammatory cytokines, chemokines and nitric oxide accompanied increased bacterial burden in the spleen. Surprisingly, no differences in *S. typhimurium* invasion of macrophages between Cav-1 null or wild type macrophages were observed.

Infections with *Pseudomonas aeruginosa* are observed most commonly in hospitalized immunocompromised individuals, resulting in urinary tract infections, pneumonia and sepsis. This is especially true of those individuals on mechanical ventilation and those suffering from severe burns. Also, over 80% of individuals with the genetic disorder Cystic fibrosis (CF) have pulmonary infection with *P. aeruginosa.*[33] Individuals with CF have a defect in the transmembrane conductance regulator (CFTR).[34,35] In normal individuals, infection with this bacteria stimulates the formation of lipid rafts that contain the CFTR thus allowing the organism to invade the respiratory epithelium and initiate inflammatory and apoptotic processes leading to shedding of bacteria-containing epithelial cells.[35–37] However, in CF patients these responses are absent and *P. aeruginosa* becomes established as a chronic infection.[38] Mechanistically, knockdown of either Cav-1, which also eliminates Cav-2, or Cav-2 alone reduces the uptake of *P. aeruginosa* into rat bronchial epithelial cells.[39] Importantly, Bajmoczi et al[40] demonstrated that following entry into host cells, *P. aeruginosa* colocalizes with Cav-1 and CFTR. More recently, increased mortality, bacterial burden and inflammation was demonstrated in Cav-1 KO compared with wild type mice, which correlated with a decreased ability of Cav-1-deficient neutrophils to phagocytose *P. aeruginosa.* Additionally, the colonization of *P. aeruginosa* was more efficient in Cav-1 KO mice. Taken together, these preclinical observations strongly suggest that Cav-1 contributes to innate immunity to *P. aeruginosa* infection, which may have clinical implications for human CF patients.[41]

PROTOZOAN PARASITES

Leishmania

Depending on the species, infection with members of the genus *Leishmania* may result in cutaneous, mucocutaneous, or visceral leishmaniasis. These diseases are found wide-spread in tropical and sub-tropical areas of the world and are major causes of morbidity and mortality. Additionally, leishmaniasis is an opportunistic infection in patients with HIV/AIDS.[42]

Leishmania spp. have a life cycle consisting of two stages, the promastigote and the amastigote. The extracellular promastigote develops in the gut of the sand fly vector until it becomes a fully virulent metacyclic promastigote. Metacyclogenesis is a process during which surface molecules associated with virulence, such as lipophosphoglycan (LPG) and MSP (called GP63), are modulated in their expression and/or posttranslational modifications.[43–45] Attainment of full promastigote virulence coincides with the feeding

cycle of the insect vector.[43–45] During a blood meal, the sand fly inoculates the parasite in the skin whereupon it is phagocytosed first by neutrophils and then by macrophages, the ultimate host cell.[46] Inside the macrophage, parasites transform from promastigotes to amastigotes over two to five days. Thereafter, amastigotes are the only form found in the mammalian host. Amastigote replication leads to the release of amastigotes from infected macrophages; amastigotes in turn are taken up by non-infected macrophages, thus spreading the infection.[47,48]

Leishmania enter macrophage phagosomes that ultimately fuse with lysosomes. The survival of *Leishmania* spp. in this hostile intracellular environment has been primarily attributed to the capacity of amastigotes to withstand the phagolysosomal compartment and to the ability of the parasite to down-modulate macrophage activation.[49–54] Accumulating evidence demonstrates that different external stimuli induce distinctive types of macrophage activation with divergent pro and/or anti-inflammatory profiles.[55–57] *L. infantum chagasi* is a cause of visceral leishmaniasis. Infection of BALB/c mouse macrophages with *L. i chagasi* promastigotes initiates a pattern of gene expression that is neither classically activated nor alternatively activated, but which demonstrates a novel type of macrophage activation characterized by an anti-inflammatory profile and an increase in the caveolae-related molecules dynamin-2, Cav-1 and Cav-3.[58] This increase in caveolae components upon *L. i. chagasi* infection suggests the presence of a feedback mechanism that may be triggered by the depletion of surface caveolae upon parasite uptake.

Employing confocal microscopy, virulent *L. i. chagasi* promastigotes were found to colocalize with the caveolae markers GM1 and Cav-1 both during entry and up to 24 hours after murine macrophage infection (Fig. 1). Entry of promastigotes via Cav-1 correlated with a delay in lysosome fusion of approximately 24 to 48 hours; a time coinciding with their promastigote-to-amastigote conversion.[48,59] Colocalization of promastigotes with Cav-1 also correlated with increased parasite survival. In contrast, serum opsonization of attenuated (avirulent) *L. i. chagasi* promastigotes precluded parasites to enter macrophages through lipid rafts/caveolae. Unlike virulent *L. i. chagasi,* avirulent parasites entered compartments that fused early with lysosomes usually within the initial three hours of infection and failed to survive in macrophages.

Because caveolae are enriched in cholesterol, transient treatment of macrophages with the cholesterol chelating agent MβCD was used to investigate the role of lipid rafts/caveolae in Leishmania infection. MβCD treatment does not affect macrophage viability if the cell membrane cholesterol is extracted while preserving the intracellular cholesterol pools.[59–61] Under these conditions, pretreatment with MβCD significantly impaired parasite entry and inhibited replication for up to 72 hours after phagocytosis, even though surface cholesterol was restored by 4 hours after treatment. Furthermore, macrophage pretreatment with MβCD accelerated the rate of lysosome fusion and led to rapid intracellular killing. Side by side comparisons showed that transient disruption of lipid rafts/caveolae caused virulent metacyclic *L. i. chagasi* to enter macrophages through a phagocytic pathway, leading to early lysosome fusion and intracellular death, resembling the entry of attenuated parasites.[59]

Surface LPG and MSP on metacyclic promastigotes are able to bind and inactivate the serum protein C3b to C3bi, facilitating parasite uptake through the macrophage receptor CR3, which localizes in caveolae.[62–65] A comparison of the receptors used by avirulent log phase versus virulent metacyclic *L. i. chagasi* in human macrophages demonstrated that metacyclic parasites preferentially enter through CR3 but not the mannose receptor and that the metacyclic parasites colocalize with Cav-1 during the initial hour of infection. In contrast, log-phase parasites ligated both the mannose receptor and CR3 and failed to colocalize with Cav-1. The ability of metacyclic, but not log phase promastigotes to

associate with CR3 and Cav-1 also correlated with a slower kinetics of lysosome fusion and increased parasite survival.[66]

The amastigote form of the parasite expresses low levels of MSP and no LPG; the latter of which inhibits lysosome fusion with promastigotes. Amastigotes can survive in the phagolysosomes and it has been suggested that the unique nutritional requirements of the amastigote can only be met in this degradative compartment.[67–69] As such, amastigotes might not benefit from entering macrophages through a cholesterol-rich/caveolae uptake mechanism that delays lysosome fusion. Indeed, side by side experiments with metacyclic promastigotes and hamster-derived amastigotes of *L. i. chagasi* supported this hypothesis. Transient depletion of cholesterol from BALB/c mouse macrophages did not affect the entry of amastigotes or their kinetics of lysosome fusion (submitted manuscript, Rodriguez, Gaur, Allen and Wilson), suggesting that the entry and survival of amastigotes is independent of cholesterol-rich microdomains including caveolae. Taken together, these results suggest that virulent, but not attenuated or log phase promastigotes, are able to exploit a caveolae-mediated pathway to facilitate their entry and intracellular survival by a mechanism that includes delayed lysosome fusion until their conversion into amastigotes and the establishment of infection.

Trypanosoma cruzi

Trypanosoma cruzi causes Chagas' disease. It is an important cause of acute myocarditis, chronic cardiomyopathy and gastrointestinal disorders in endemic areas.[70] Chagas' disease is found in endemic areas of Mexico, Central and South America. In recent years, there has been an increased recognition of Chagas' disease among immigrants from endemic areas into North America and Europe. Chagas' disease is also an opportunistic infection in patients with HIV/AIDS.[71–73]

The parasite has a complex life cycle. During a blood meal from an infected mammalian host, the insect vector ingests blood-form trypomastigotes, which undergo transformations and after 3 to 4 weeks, infective, nondividing metacyclic trypomastigotes are present in the hindgut of the vector and are deposited with the feces of the vector during subsequent blood meals. Transmission to the new host occurs when the parasite-laden feces contaminate oral or nasal mucous membranes, the conjunctivas, or other vulnerable surfaces. Other modes of transmission include blood transfusion, organ donation, congenital, breast milk, ingestion of contaminated food or drink and laboratory accident.

When the trypomastigotes enter a host cell they transform into amastigotes where they multiply by binary fission and again transform to trypomastigotes. Trypomastigotes are released as the host cell ruptures and disseminate through the lymphatics and the bloodstream to find new cells to invade. The precise mechanism(s) by which the parasite enters the host cell is not entirely understood, however several receptors have been implicated in this process. Although any nucleated mammalian cell can be parasitized, those of the cardiovascular system, including cardiac myocytes, cardiac fibroblasts, endothelial cells and vascular smooth muscle cells, as well as cells of the reticuloendothelial, nervous and muscle systems and adipose tissue, appear to be favored.

T. cruzi infection is characterized by an intense inflammatory reaction accompanied by an upregulation of cytokines and chemokines.[73,74] Pathological examination of the cardiovascular system in both human samples and experimental acute chagasic myocarditis reveals inflammation, myonecrosis, vasculitis and numerous parasite pseudocysts. In chronic chagasic cardiomyopathy, inflammation, fibrosis, myocytolysis and vasculitis persist but there are few parasites in the infected tissues. In many chronically infected individuals there is a dilated cardiomyopathy. In order to understand the mechanisms

involved in progression of disease and the development of the resulting cardiomyopathy, many groups have focused their research either on the in vitro infection of cardiac cells with *T. cruzi* or through the use of murine models of infection.

Caveolin is a negative regulator of extracellular signal-regulated kinases (ERK) and cyclin D1.[75] Thus, a reduction in the expression of Cav-1 and Cav-3 generally results in the upregulation of ERK activity and an induction of *cyclin D1* expression, which contribute to cardiac myocyte hypertrophy and ultimately cardiomyopathy. Interestingly, Cav-1 and Cav-3 null mice as well as the Cav-1/Cav-3 double null mice display a cardiomyopathic phenotype associated with cardiac myocyte hypertrophy and interstitial fibrosis.[76–78] Thus, it was of great interest that during the acute phase of *T. cruzi* infection a reduction in the expression of Cav-1, Cav-2 and Cav-3 was observed. This was accompanied by activation of ERK, activator protein 1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and increased cyclin D1 expression.[79,80] The change in Cav-1 expression in infected mice was the result in part of infection of the cardiac fibroblasts since cardiac myocytes do not express Cav-1. At 60 days post-infection, which is considered the sub-acute/chronic phase, there was a reduction in Cav-3 expression which normalized by day 180 post-infection,[81] though the increase in the expression of ERK persisted. Thus, it would appear that the initial reduction in Cav-3 expression may trigger an increase in ERK, leading to cardiac myocyte hypertrophy.

Chagas' disease is also a vasculopathy and in carotid arteries obtained from *T. cruzi* infected mice there was a reduction in the expression of Cav-1 and Cav-3 and activation of ERK, cyclin D1 and endothelin-1(ET-1).[82] These findings may in part explain the vasoconstriction observed as a result of this infection. *T. cruzi* infection also results in a reduction of Cav-1 and Cav-2 expression and an increase in activated ERK, cyclin Dl and ET-1.[82,83] In addition, infection of cardiac myocytes results in a reduction of Cav-3 expression and a concomitant increase in activated ERK (Fig. 2).[81] Taken together *T. cruzi* infection results in cardiomyopathy and a vasculopathy in which caveolin, ERK, cyclin D1 and ET-1 contribute to cardiovascular remodeling and the pathogenesis of chagasic heart disease.

Employing the highly virulent Tulahuen strain of *T. cruzi* infection, Medina et al[84] infected both Cav-1 wild type and Cav-1 null mice and while the resulting mortality was 100% in both genetic backgrounds, death was slightly delayed in the wild type mice. The parasitemia in the Cav-1 null mice was significantly reduced compared with wild type mice. In both groups there were numerous pseudocysts, myonecrosis and marked inflammation. Interestingly, infection of cultured cardiac fibroblasts obtained from Cav-1 null and wild type mice revealed no differences in infectivity. Determination of serum levels of several inflammatory mediators revealed a significant reduction in IFN-γ, TNF-α and components of the nitric oxide pathway in infected Cav-1 null mice, while infection of wild type mice resulted in an increase in these inflammatory mediators. The defective production of chemokines and cytokines observed in vivo is, in part, attributed to Cav-1 null macrophages. These results suggest that Cav-1 may play an important role in the normal development of immune responses. Recently, Barrias et al demonstrated that infection of mouse peritoneal macrophages with the Y strain of *T. cruzi* was impaired when cells were treated with MβCD.[85] The contributions of caveolin in Chagas' disease are not entirely understood and investigators are continuing to explore these interactions.

Toxoplasma gondii

Toxoplasma gondii is a ubiquitous Apicomplexan obligate intracellular protozoan parasite of mammals and birds. It has long been recognized as being an important cause of congenital infection with chorioretinitis and central nervous system manifestations and has also emerged as an opportunistic pathogen in immune compromised hosts where it primarily

causes encephalitis.[86–88] Although overwhelming disseminated toxoplasmosis has been reported, the predilection of this parasite for the central nervous system causing necrotizing encephalitis and the eye causing chorioretinitis constitutes its major threat to patients. The development of these diseases is a consequence of the transition of the resting or latent bradyzoite stage to the active rapidly replicating form, the tachyzoite stage.[86–87,89–93] It is likely that in chronic toxoplasmosis tissue cysts (bradyzoites) regularly transform to tachyzoites and that these active forms are removed or sequestered by the immune system, while some invade host cells differentiating to new tissue cysts.[86,89,90,93]

T. gondii replicates within a parasitophorous vacuole, isolated from host vesicular traffic.[94] *T. gondii* are capable of actively invading host cells[95,96] and these invasion processes require parasite motility, orientation toward the host cell and sequential discharge of three secretory organelles termed micronemes, rhoptries and dense granules.[97,98] Host cell cholesterol is required for entry and intracellular replication of this and other pathogens.[99–102] Central roles for cholesterol are suggested at the attachment, penetration and intracellular multiplication stages. Depletion of membrane cholesterol leads to a loss of invaginated caveolae.[103] The predominant sterol in *T. gondii* membranes is cholesterol and it has been unequivocally demonstrated that this parasite is auxotrophic for this major lipid.[101] *T. gondii* actively intercepts low-density lipoprotein (LDL)-derived cholesterol that has transited through host lysosomes by a yet undefined mechanism. Cholesterol trafficking from mammalian lysosomes to intravacuolar *T. gondii* requires functional host Niemann–Pick type cholesterol proteins (NPC), which are known to mediate cholesterol egress across the endosomal (mainly NPC1) and lysosomal (mainly NPC2) membranes,[104] and trafficking is independent of pathways involving the host Golgi and endoplasmic reticulum.[101] The uncoupling between LDL uptake and cholesterol biosynthesis occurring during *T. gondii* infection[101] leads to the assumption that these pathways are dramatically perturbed in infected cells.

Caveolae/caveolins have been proposed to function in a number of cholesterol-trafficking steps. These steps include selective cholesterol uptake from HDL via the scavenger receptor SR-B1 located in caveolae (cholesterol influx) and the delivery of newly synthesized cholesterol from the endoplasmic reticulum membrane caveolae, where it is delivered to HDL (cholesterol efflux).[105] However, studies have shown that high levels of host caveolae vesicles are not needed for trafficking of LDL-derived lysosomal cholesterol to Toxoplasma in mammalian cells.[101] It has been suggested that a probable scenario during *T. gondii* infection is that following LDL receptor-mediated endocytosis, cholesterol is liberated from LDL cholesteryl ester in early hydrolytic compartments containing the enzyme acid lipase. The cholesterol then effluxes from the NPC-containing late endosome/lysosome before trafficking to the parasitophorous vacuole. The post-endolysosomal movement of cholesterol to the parasitophorous vacuole is blocked by inhibitors of vesicular transport but does not require vesicle fusion or host endolysosome fusion with the parasitophorous vacuole. Transit to the parasitophorous vacuole is direct, rather than across host organelles, e.g., endoplasmic reticulum, Golgi[101,106] or through the host plasma membranes.[107] However, the parasitophorous vacuole is accessible to sterol acceptors in the medium by an unknown mechanism. Neither the host sterol carriers SCP-2 nor caveolins are involved and the process is independent of vimentin intermediate filaments. Host cholesterol is delivered to the parasitophorous vacuole via lipid extractor-or transporter-like proteins on the parasitophorous vacuole membrane, then trafficked within the vacuolar space, perhaps in association with the tubulo-vesicular network (TVN) secreted by *T. gondii* before internalization into the parasite interior via parasite plasma membrane proteins and storage as cholesteryl esters in lipid bodies.

Rhoptries are elongated club-shaped organelles containing a densely packed granular material in their basal bulbous portion and are related to secretory lysosomes or exosomes.[108] *T. gondii* rhoptries are formed via the endocytic pathway[109] and contribute to the formation of the *Toxoplasma* and *Plasmodium* parasitophorous vacuole membranes (PVMs) by releasing their contents from the anterior end of the parasite during invasion.[98,110,111] *Toxoplasma* rhoptries also contain lipids, including large amounts of cholesterol and phosphatidylcholine.[112,113] In these organelles, the cholesterol/phospholipid molar ratio (1.5/1) is too high for lipid bilayer stability, suggesting that some rhoptry cholesterol molecules may be organized in a crystalline array inside the organelle. Although it is plausible that rhoptry cholesterol is incorporated into the PVM during invasion and that rhoptry discharge can effectively compensate for the absence of caveolae in host cells, this possibility requires testing.

The biogenesis of the PVM surrounding *Toxoplasma* is only partially understood. Capacitance measurements in patch-clamped host cells indicate that at least 80% of the membrane in the nascent vacuolar membrane is host cell derived.[114] This is consistent with observations showing that fluorescent tracers inserted into the host plasma membrane before infection were incorporated into the nascent PVM of *Toxoplasma*[110,115,116] as well as into the *Plasmodium* PVM.[117] Additionally, multiple lines of evidence suggest that rhoptry contents contribute directly to formation of the vacuolar membrane.[109,110,118–120] These apparently conflicting observations can be resolved by postulating a two-step process of invasion. Initial discharge of the rhoptry contents directly into the host cytoplasm leads to coalescence of multivesicular structures, which then fuse with the nascent vacuole that is derived primarily from the host cell plasma membrane.[121] Whether and how cholesterol contributes to this unusual process of cell invasion by *T gondii* is not clear. Studies have evaluated the ability of cholesterol-depleted parasites to invade normal cells, of cholesterol-depleted cells to be invaded by untreated parasites, as well as the ability of normal parasites to invade caveolin-minus cells. The results demonstrate that *T gondii* is dependent upon host plasma membrane cholesterol to trigger organelle discharge. Neither rhoptry-derived cholesterol nor caveolae microdomains in the host cell plasma membrane are required to complete invasion. These results identify a heretofore unexpected mechanism by which cholesterol regulates microbial entry into mammalian cells.

Lisanti and colleagues[122,123] discovered that purified caveolae microdomains contained an abundance of signaling molecules, such as Src-like tyrosine kinases and heterotrimeric G proteins and proposed that Cav-1 and caveolae may serve as docking points for numerous cell surface receptors, which, when activated by ligand binding, are recruited to caveolae.[122,123] It has been demonstrated that GTPase activity of G protein-subunits could be suppressed by a peptide derived from the NH2 terminus of Cav-1 termed the caveolin scaffolding domain, demonstrating the interdependence of these proteins for functional activity.[124] Finally, studies have shown that interaction of the *T. gondii*-derived molecule cyclophillin-18 (C-18) with a chemokine receptor (CCR5) on dendritic cells is critical for IL-12 (a pro-inflammatory mediator) induction in vivo and the subsequent control of parasite growth.[125] Since CCR5 is associated with G-protein-subunits, it is interesting to consider the possible participation of caveolin in the signaling pathways and transcriptional events downstream of CCR5 triggered by *T gondii.*

FUNGI

Although significant efforts have led to an appreciation that sterol-rich membrane domains significantly impact the function of fungal membranes,[126] there is limited information regarding the role of lipid rafts and caveolae in the pathogenesis of fungal disease in mammalian hosts. The most in depth analysis of a fungal interaction with cellular membrane

rafts comes from a study on *Paracoccidioides brasiliensis,* a dimorphic fungus endemic in the environment in Central and South America.[125] This fungus causes a spectrum of human disease ranging from mild localized infection to disseminated deep lesions, characterized by granulomatous inflammation. *P. brasiliensis* is frequently acquired via inhalation. Using epithelial alveolar cells, Maza et al[127] demonstrated that host cell lipid rafts are critical for engaging *P. brasiliensis* cells. They demonstrated that ganglioside GM1 appeared to be recruited to the point of adhesion of *P. brasiliensis* to this host cell. Furthermore, pretreatment of the alveolar cells with either MβCD to deplete host cell cholesterol, or nystatin, to bind host cholesterol, significantly reduced fungal adhesion.

Pneumocystis species are frequent pulmonary pathogens in hosts with compromised cellular immune responses, especially individuals with AIDS and the fungus can intimately engage Type I alveolar epithelial cells.[128] Although *Pneumocystis* associates with cells, the fungus is an extracellular pathogen. Nevertheless, the fungus interdigitates with the alveolar cells and activates caveolae of the host cells, resulting in the close proximity of plasmalamellar vesicles in the areas of contact. Presumably, this allows the fungus to parasitize the host cells for nutrients.

Beta-glucans are major components of many fungal cell walls including that of *Candida albicans,* which is the most prevalent cause of systemic mycoses. Beta-glucans from *C. albicans* can interact with very long fatty acid-containing lactosylceramide lipid rafts on the plasma membrane of human neutrophils, inducing migration of the neutrophil toward the fungus[129,130] and, presumably, enhancing phagocytosis of the yeast.

Histoplasma capsulatum, a dimorphic fungus endemic to the Mississippi and Ohio River Valleys of the USA and in regions within Central and South America, causes pulmonary and disseminated infections, especially in individuals with compromised immunity. Calcium binding protein (CBP) is a well known virulence factor for the fungus that recently was shown to have structural homology with mammalian saposin B.[131] Hence, it has been proposed that CBP may interact with host glycolipids, including those present in caveolae. This finding underscores the probable broad and yet undiscovered import of caveolae in interactions with pathogenic fungi. Future work will continue to elucidate the functional importance of caveolae interactions with fungi during adhesion, internalization and internal processing.

CONCLUSION

A growing list of pathogens, including viruses, bacteria and their associated toxins, fungi and even prions, can interact with caveolae membrane domains.[2] The intracellular trafficking of these agents via caveolae differs dramatically from the usual route of ligands internalized by clathrin-mediated endocytosis. The use of caveolae for cellular entry allows the pathogen to avoid classical endosome-lysosome trafficking and, consequently, avoid degradative compartments within the cell.

Acknowledgments

The work was supported by CNPq and FAPEMIG (FSM), NIH grants AI- 076248 (HBT) NIH Grants AI39454 (LMW) and AI045540 (MEW and Veterans Affairs Merit Review (MEW). Daniel Adesse was supported in part by a Fogarty Training Grant D43 TW007129 (HBT). This work was also supported in part by a Career Developmental Award (CDA-2) from the Departments of Veterans Affairs (NER).

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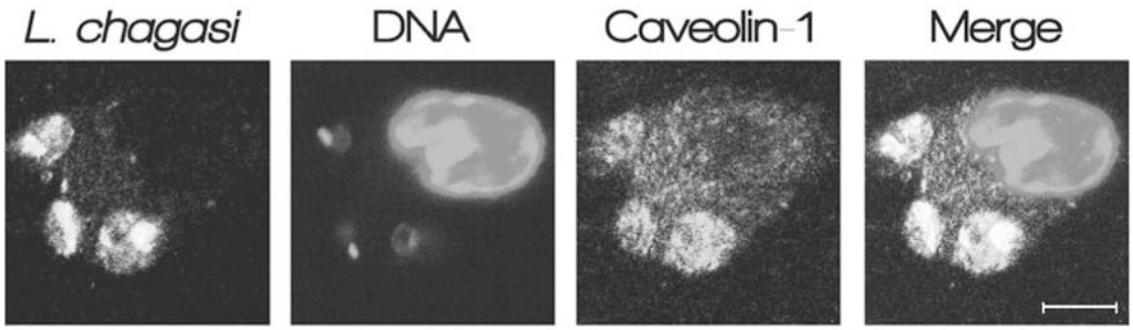

Figure 1.

Intracellular *L. i. chagasi* colocalizes with caveolin-1. Bone marrow macrophages were infected with carboxy-fluorescein diacetate succinimidyl (CFSE) labeled *L. i. chagasi* promastigotes (green). Macrophage and parasite DNA was stained with TO-PRO-3 (blue). Confocal microscopy was used to assess colocalization of markers at serial time points. Caveolin-1 (red) clustered at the entry site of *L. i. chagasi* promastigotes and remained associated with parasites for 24 to 48 hours after infection. Shown is a picture taken at 24 hours of infection. Scale bar: 5 μm. A color version of this image is available online at www.landesbioscience.com/curie. Reproduced with permission from Adesse et al. Cell Cycle 2010; 9:1639–1649.[81]

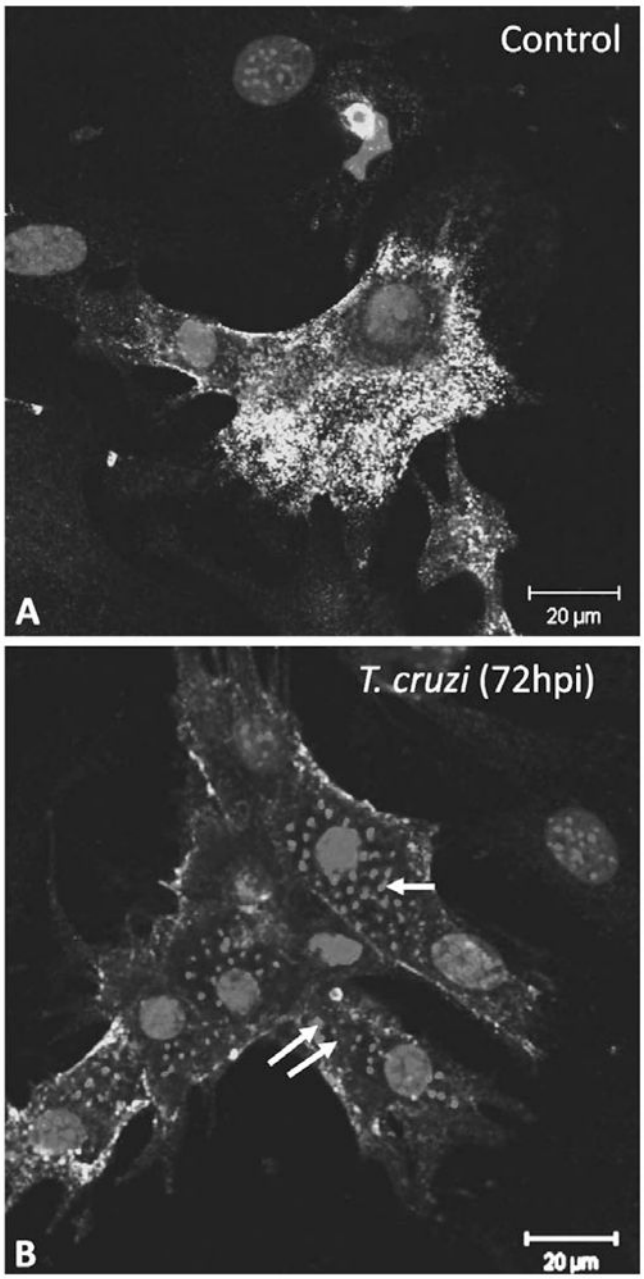

Figure 2.

Caveolin-3 (Cav-3) expression is diminished after *Trypanosoma cruzi* infection: Cardiac myocytes were isolated from mouse embryos and infected with trypomastigotes. Confocal microscopy showed that uninfected cultures displayed abundant Cav-3 immunoreactivity (A), including peripheral staining. Cav-3 signal was reduced among highly parasitized myocytes (B) and was present predominantly at the cell periphery. DNA staining by DAPI permitted visualization of host cell nucleus and the amastigote kinetoplastid DNA. Bars = 20 mm. Reproduced with permission from Adesse et al. Cell Cycle 2010; 9:1639–1649.[81]

Table 1

Summary of papillomavirus entry pathways

HPV Type	Pathway Identified	Methods Used	Ref.
BPV1	clathrin mediated entry, shuttling from endosomes to caveosomes	biochemical inhibitors, colocalization studies, caveolin1 shRNA, dominant negative Cav-1, 293 cells, pseudovirions	27
BPV1/HPV16	clathrin mediated entry	biochemical inhibitors, colocalization studies, C127 cells, virions and VLPs	19
HPV16	clathrin/caveolae independent, lipid raft independent, dynamin independent, tetraspanins involved	siRNA KO of clathrin, cav1, dynamin and tetraspanins, biochemical inhibitors, caveolin −/− cells, dominant negative inhibitors 293TT and HELA cells, pseudovirions	23
HPV16	clathrin mediated entry, shuttling from endosomes to caveosomes	biochemical inhibitors, colocalization studies, caveolinl shRNA, HaCaT cells, pseudovirions	26
HPV16/HPV31	clathrin mediated entry (HPV16), caveolar uptake (HPV31)	biochemical inhibitors, dominant negative inhibitors, HaCaT cells, pseudovirions	22
HPV16/HPV31	clathrin mediated entry	biochemical inhibitors, 293TT and COS7 cells, pseudovirions	20
HPV31	Caveolar mediated uptake, Rab5 mediated shuttling to endosome	biochemical inhibitors, colocalization studies, dominant negative Rab5, HaCaT cells, pseudovirions	28
HPV16/31 and 58	clathrin mediated entry (HPV16 and 58) caveolar uptake (HPV31)	biochemical inhibitors colocalization studies, COS7 cells, VLPs and pseudovirions	18
HPV33	noncaveolar uptake	biochemical inhibitors, COS7 and HELA cells, pseudovirions	21