The role of lipid rafts in the pathogenesis of bacterial infections

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Received 11 May 2005; received in revised form 9 October 2005; accepted 11 October 2005
Available online 26 October 2005

Abstract

Numerous pathogens have evolved mechanisms of co-opting normal host endocytic machinery as a means of invading host cells. While numerous pathogens have been known to enter cells via traditional clathrin-coated pit endocytosis, a growing number of viral and bacterial pathogens have been recognized to invade host cells via clustered lipid rafts. This review focuses on several bacterial pathogens that have evolved several different mechanisms of co-opting clustered lipid rafts to invade host cells. Although these bacteria have diverse clinical presentations and many differences in their pathogenesis, they each depend on the integrity of clustered lipid rafts for their intracellular survival. Bacterial invasion via clustered lipid rafts has been recognized as an important virulence factor for a growing number of bacterial pathogens in their battle against host defenses.

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Keywords: Bacteria; Lipid raft; Caveolae; Caveolin

1. Introduction

A wide range of pathogens including parasites, viruses, and bacteria have evolved mechanisms of invading host cells in order to survive, and replicate [1]. Many of these organisms co-opt host endocytic pathways in order to invade phagocytic and non-phagocytic cells and thereby avoid host defenses [2]. The different routes of entry used by these pathogens have a profound effect on their pathogenesis. Even after invasion of host cells, pathogens may still not be safe from host defenses. Intracellular pathogens must also find a way to avoid degradation in the acidic environment of intracellular lysosomes. Several pathogens have the ability to enter host cells via the classical endosome–lysosome route involving clathrin-coated pits but then avoid destruction within lysosomal compartments either by preventing fusion with lysosomes or alternatively, preventing acidification following fusion with lysosomal compartments [2–4]. In addition to the endosome–lysosome pathway, both phagocytic and non-phagocytic cells have been recognized to have alternative pathways of endocytosis [2,4]. It has recently been recognized that an increasing number of pathogens co-opt the endocytic properties of caveolae or lipid raft to achieve entry into host cells [1,3,5]. Lipid rafts are plasma membrane domains that are composed of cholesterol, glycosphingolipids, and GPI anchored proteins. Although initially described as plasma membrane invaginations, caveolae are defined as caveolin-enriched lipid rafts and are recognized to take a variety of shapes including flat, tubular, or vesicular [6]. Caveolin proteins are not only found in the cell membrane, but they can also be found intracellularly as part of vesicle membranes, the endoplasmic reticulum, or free within the cytosol [7,8]. Clustered lipid rafts are made up of an assembly of lipid raft units which have been implicated in a variety of cellular functions including signaling and growth regulation [9]. In addition, clustered lipid rafts are believed constitute an alternate pathway of endocytosis, often distinct from clathrin-coated pits [9]. However, it has recently been recognized that these pathways may also overlap as certain raft structures are internalized via a clathrin-dependent mechanism [10,11]. After endocytosis clustered lipid rafts can form intracellular vesicles that may have a variety of fates including remaining as an intracellular organelle or a caveosome, fusing with the endoplasmic reticulum or Golgi network, as well as transcytosing to the contralateral cell surface [12–14]. Clustered lipid rafts are believed to play an important physiological role in macromolecular transport [9]. The ability of pathogens to exploit this endocytic pathway may be a key to understanding the pathogenesis of these diverse infections. Pathogens that are
endocytosed via clustered lipid rafts do not generally fuse with traditional lysosomes and the contents are targeted to a range of different intracellular compartments (Fig. 1) \[6,15\]. Thus, lipid raft-mediated uptake may promote intracellular survival and dissemination within the host.

A wide variety of bacterial pathogens have evolved mechanisms of co-opting host endocytic pathway via clustered lipid rafts (Table 1). While an increasing number of pathogens are now recognized to co-opt lipid raft-mediated endocytosis as a means of invading host cells including both viruses and bacteria, this review will focus on a few examples where the role of clustered lipid rafts in promoting bacterial internalization have been better defined. The role of lipid rafts in the adherence and uptake of bacterial toxins plays an important role in a number of bacterial infections including shigellosis and anthrax \[10,16,17\]. An outstanding review of this topic can be in the review by Lafont and van der Goot \[18\]. In addition to bacterial uptake, this review will focus on the role of some of the key lipid raft associated proteins including caveolin proteins that have recently been recognized to play a role in regulating bacterial internalization. Although each of these pathogens require clustered lipid rafts for their intracellular survival, it is important to recognize that these structures and mechanisms are not identical. Different pathogens co-opt lipid rafts during distinct different sections of their pathogenesis. In addition, lipid rafts are composed of a group of heterogeneous structures and the exact composition of these lipid rafts varies among both cell types and pathogens. A detailed description of the molecular biology that distinguishes these unique structures

![Fig. 1. Schematic diagram showing the complex role of caveolae and lipid rafts in bacterial pathogenesis. Bacterial that co-opt this endocytic pathway are able to survive intracellularly and avoid fusion with traditional lysosomes. These bacteria can use caveolae or lipid raft-mediated endocytosis and be targeted to a number of different intracellular compartments. Bacterial entry through lipid rafts also allows alteration of cell signaling pathways including inflammatory cytokine production and induction of apoptosis.](image-url)

<table>
<thead>
<tr>
<th>Bacterial pathogens that co-opt lipid raft or caveolae-mediated endocytosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>[55,66]</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>[38,46]</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>[71,72]</td>
</tr>
<tr>
<td>Chlamydia species</td>
<td>[67,69]</td>
</tr>
<tr>
<td>Mycobacteria species</td>
<td>[83–85]</td>
</tr>
<tr>
<td>Brucella species</td>
<td>[75,77,81]</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>[88]</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>[16]</td>
</tr>
<tr>
<td>Ehrlichia chaffeensis</td>
<td>[89,90]</td>
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<tr>
<td>Anaplasma phagocytophilum</td>
<td>[89,90]</td>
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<tr>
<td>Group A Streptococci</td>
<td>[91]</td>
</tr>
</tbody>
</table>

Although these bacterial pathogens have many differences in both their clinical presentation and bacterial pathogenesis, they each have evolved mechanisms to usurp normal host endocytic pathways as a means of invasion. The mechanism of invasion for each of these diverse bacterial pathogens depends on the integrity of clustered lipid rafts.
is beyond the scope of this review. The examples provided below detail the mechanism by which bacterial pathogens have evolved the mechanism to co-opt cholesterol rich, detergent insoluble membrane domains that as a group have been termed clustered lipid rafts. Studying the role of lipid rafts in the pathogenesis of these bacterial infections has allowed us great insight into the pathogenesis of the diverse diseases as well as better understanding into the role of lipid rafts in normal cellular physiology.

2. Caveolin proteins

Caveolin proteins are key components of lipid rafts and caveolae and have many important functions. Caveolin was originally described in 1992 as Vesicular Integral Membrane protein of 21 kDa (VIP21) [19]. VIP21 was initially identified in epithelial cells as key molecule of vesicular transport. VIP21 was able to be identified in the Golgi apparatus, the plasma membrane, as well as intracellular vesicles [19]. Later VIP21 was renamed caveolin-1 and continues to be recognized as an integral protein of caveolae [20,21]. The caveolin family of proteins is made of 3 proteins: caveolin-1, caveolin-2, and caveolin-3. While caveolin-1 and -2 have been identified in most cell types, the expression of caveolin-3 is restricted to muscle [6].

While caveolae have been implicated in a number of different cellular functions, the exact roles of caveolin-1 are unclear. The amino and carboxy terminal of caveolin-1 resides in the cell cytoplasm whereas the transmembrane domain of the molecule is associated with the lower leaflet of the plasma membrane forming a hairpin structure [19,20]. Caveolin-1 proteins interact to form homo-oligomers ranging from 300 to 400 kDa. Because of their ability to form stable homo-oligomers, caveolin-1 has a role in determining the size and shape of certain caveolae [22]. Oligomerization occurs in the endoplasmic reticulum immediately after the protein in synthesized [23]. The oligomerized form of caveolin-1 is associated with lipid rafts in both the Golgi and the plasma membrane. Some of these oligomers create more stable forms which are resistant to disassociation in the presence of 2% SDS. Electron microscopy has revealed that caveolin-1 aggregates form striated filamentous strands which coat the cytoplasmic face of cave like plasmalemmal caveolae [23]. Caveolin-1 has also been implicated as a scaffolding protein for organizing and concentrating caveolin-interacting signaling molecules within caveolae [24]. Recently, studies of SV40 uptake via caveolae have identified that caveolar domains initially form in the Golgi apparatus. In addition, there is little exchange of caveolin-1 between difference these caveolar domains which appear to serve a stable, fixed vesicular transport containers [25]. These vesicles can transport cargo to multiple different compartments and even intersect with more traditional endocytic pathways. Stable caveolar vesicles have been shown to transiently fuse with the early endosome and release their contents in a pH-dependent manner [26]. It has also been shown that phosphorylation of caveolin-1 can trigger the flattening, aggregation, and intracellular fusion of caveolae and caveolae-derived vesicles [27]. In addition to serving as key structural proteins that organize caveolae platforms, caveolin proteins are important in regulating endocytosis and cell signaling [28,29].

Caveolin-2 is the least well studied of the caveolin gene family and the role of caveolin-2 in lipid raft-mediated endocytosis is unknown. Caveolin-1 and -2 are co-expressed on most cell types including high levels of expression on the endothelium as well as type I pneumocytes within the alveolar epithelium [30–32]. Caveolin-2 is the most divergent of the caveolin gene family with only approximately 50% similarity to caveolin-1 [33]. Caveolin-2 interacts with caveolin-1 to form a hetero-oligomeric complex within lipid rafts [34]. Unlike caveolin-1, caveolin-2 is not required for the formation of caveolae and cannot form caveolae by itself [34,35]. In addition, caveolin-2 has not been implicated as an important regulator of endocytosis or host cell signaling pathways [31,36]. In order for caveolin-2 to be expressed, caveolin-1 is required as a chaperone to transport caveolin-2 to the cell surface [37]. In the absence of caveolin-1, caveolin-2 is degraded in the Golgi apparatus [34,37]. This dependence of caveolin-2 on caveolin-1 expression has confounded the studies aimed at distinguishing the function of these two key lipid raft associated proteins. In several of the following examples of bacterial invasion, we highlight examples where the complex roles played by both caveolin-1 and -2 in lipid raft-mediated endocytosis are just beginning to be understood.

2.1. Escherichia coli

*E. coli* was one of the first bacterial pathogens recognized to invade host cells via clustered lipid rafts [38]. *E. coli* is the most common cause of urinary tract infections as well as a frequent cause of extraintestinal infections [39]. Like many other gram-negative bacteria, *E. coli* possess fimbriae or pili. The major virulence factors expressed by uropathogenic *E. coli* is the FimH adhesin, a mannos-binding lectin on the tips of bacterial fimbrial appendages. FimH allows the bacteria to bind to cells and colonize mucosal surfaces [40,41]. *E. coli* was traditionally considered an extracellular pathogen, but has increasingly been recognized to invade multiple cell types. It was initially discovered that FimH expressing *E. coli* were internalized by macrophages and mast cells [38,42]. These bacteria were able to survive and replicate within intracellular vesicles and avoid fusion with lysosomes. In contrast, *E. coli* that were opsonized with antibodies were internalized by a different mechanism, trafficked to acidic lysosomes, and rapidly degraded. Opsonization has been shown for other pathogens to have similar effects in determining the mechanism of uptake. The unopsonized pathogens enter via clustered lipid rafts and are able to survive intracellularly, whereas the opsonized pathogens are uptaken via traditional clathrin-coated pits and degraded with intracellular lysosomes [43]. Subsequently, the receptor for FimH expressing *E. coli* was identified as a glycosylphosphatidylinositol (GPI) anchored protein, CD48 [38]. Since GPI linked proteins are predominantly located within lipid rafts, this discovery gave the first hint of the
involvement of lipid rafts in bacterial invasion. Disruption of lipid rafts by using cholesterol depleting agents such as methyl-β-cyclodextrin, filipin or nystatin markedly decreased the entry of FimH expressing E. coli. On the other hand, cholesterol disrupting agents had no effect on the uptake of antibody-coated E. coli [38]. Thus, a traditionally extracellular pathogen, E. coli, is able to invade macrophages and mast cells by co-opting the host pathway of lipid raft-dependent endocytosis. These series of observations were the first description of this novel bacterial pathway for invasion via clustered lipid rafts.

Mucosal surfaces of the respiratory, gastrointestinal and genitourinary tracts represent the major sites of initiating microbial infections; therefore, it was important to determine if E. coli can invade epithelial cells via similar mechanisms. The mucosal epithelium performs an important barrier function and is not typically amenable to bacterial invasion. The bladder epithelium is also especially highly impermeable to invasion because of the role of the bladder as a storage organ for urine. The presence of “plaques” comprising of uroplakin proteins localized on the luminal surface of superficial bladder and their association with clusters of cholesterol and sphingolipids is believed to contribute to a more ordered lipid structure in the plasma membrane, lowering membrane fluidity and permeability. The virulence of E. coli in the bladder has also been linked to the expression of FimH. While FimH binds CD48 on mast cells and macrophages, FimH binds to uroplakin-1a on the plasma membranes of bladder cells. FimH binding to uroplakin promotes not only bacterial attachment but also bacterial invasion of these relatively impermeable cells [44,45]. The involvement of clustered lipid rafts in the invasion of the bladder epithelium by E. coli was deduced from the observation that the FimH receptor, uroplakin 1a, was specifically localized to lipid raft fractions of bladder cell extract [46]. Disruption of lipid rafts with cholesterol depleting agents has been shown to inhibit E. coli invasion of bladder epithelial cells both in vitro and in vivo. In addition, by using confocal microscopy it has been shown that intracellular E. coli are surrounded by vesicles enriched in lipid raft components including GM1, cholesterol and caveolin-1 [46].

Caveolin-1 has been implicated as a key structural component of caveolae that interacts with both the cytoskeleton [47] as well as key signaling molecules [48,49] and may be a critical regulator of bacterial uptake via clustered lipid rafts. Caveolin-1 appears to be critically important for E. coli entry into bladder epithelial cells. Decreased expression of caveolin-1 by using RNA interference markedly decreased the ability of E. coli to invade bladder epithelial cells [46]. In contrast, caveolin-1 expression appears to downregulate uptake of proteins such as Autocrine Motility Factor (AMF-1) [50]. It has been proposed that caveolin-1 expression may decrease membrane fluidity and therefore decreased caveolin-1 expression can lead to increased caveolae-mediated uptake of specific proteins. In contrast, uptake of large bacterial pathogens such as E. coli via clustered lipid rafts is dependent on caveolin-1 expression. It is possible that caveolin-1 is required for formation of the large platforms that control lipid raft-dependent bacterial uptake or alternatively caveolin-1 expression could exert its effect via changes in cell signaling cascades. No longer considered predominantly an extracellular pathogen, an important virulence factor of E. coli is the ability to invade host cells via clustered lipid rafts and is dependent on membrane cholesterol as well as caveolin-1 expression.

2.2. Pseudomonas aeruginosa

Pseudomonas aeruginosa is an important cause of nosocomial pneumonia, as well as a major pulmonary pathogen in patients with cystic fibrosis and other immunocompromising conditions [51–54]. Pseudomonas was initially shown to invade both nasal and bronchial epithelial cells via ceramide rich lipid rafts [55]. It has been proposed that Pseudomonas stimulates lipid raft associated receptors and induces transformation of small lipid rafts into large insoluble membrane platforms [55]. For example, stimulation of CD95 and CD40 induces this membrane reorganization by exposing the enzyme acid sphingomyelinase on the extracellular leaflet of the plasma membrane and converting sphingomyelin to ceramide [56]. Pseudomonas infection of airway epithelial cells has been shown to induce the formation of these large ceramide rich lipid rafts which play a critical role in mediating bacterial invasion [55]. Removal of membrane cholesterol with methyl-β-cyclodextrin inhibits both ceramide rich raft formation and Pseudomonas invasion of nasal and airway epithelial cells both in vitro and in vivo [55,57].

Ceramide rich lipid rafts have been implicated to serve many roles in the pathogenesis of Pseudomonas infections including the regulation of apoptosis and cytokine production. Ceramide rich lipid rafts are important in clustering cell surface receptors and cell signaling proteins [56]. Pseudomonas upregulates CD95 expression within lipid rafts via its type III secretion system. Pseudomonas infection leads to ligation of the CD95 receptor and induces epithelial cell apoptosis. Disruption of lipid rafts with methyl-β-cyclodextrin inhibits the CD95-dependent apoptosis. In addition, mice lacking CD95 have an increased susceptibility to Pseudomonas infections [55]. Therefore, it appears that both Pseudomonas invasion and CD95-dependent apoptosis of bronchial epithelial cells is dependent on the integrity of lipid rafts and a crucial host defense mechanism to Pseudomonas infection.

Pseudomonas infection of upper airway epithelial cells leads to the production of inflammatory cytokines including IL-1β. Lipid rafts are believed to play an important role in regulating cytokine production due to their ability to organize cell surface receptors and cell signaling molecules in specialized domains. One model has proposed that the chloride channel CFTR, which is defective in Cystic Fibrosis, may serve as a cell surface receptor for Pseudomonas [57]. CFTR clusters into lipid rafts only after Pseudomonas infection and co-localizes with the lipid raft marker GM1 and Pseudomonas. Disruption of lipid rafts with methyl-β-cyclodextrin inhibits migration of wild type CFTR proteins and mutant forms of the CFTR protein are unable to localize to the lipid raft domains on the plasma membrane. Pseudomonas infection via CFTR induces NF-KB activation and the induction of cytokine
production. Methyl-\(\beta\)-cyclodextrin treatment also inhibits this activation of NK-KB that is believed to play a role in the host defense against *Pseudomonas* [57]. In contrast, disruption of lipid rafts prior to *Pseudomonas* infection of epithelial cells leads to markedly increased levels of IL-1B and a greater inflammatory response [55]. Lipid rafts appear to be important regulators of cytokine production in response to *Pseudomonas* possibly via uptake of bacteria which may interfere with gene transcription or protein translation. Alternatively, the organization of lipid rafts may be required for alignment of key regulatory molecules. Instead of protecting the host by enhancing bacterial clearance, this exaggerated inflammatory response after disruption of lipid rafts directly leads to increased morbidity and mortality from uncontrolled inflammation [55]. While invasion via lipid rafts appears to offer an advantage to the bacteria by avoiding host defenses, it is possible that lipid raft-mediated invasion of *Pseudomonas* in the upper airways may actually be protective to the hosts. Lipid rafts play a key role in cell signaling events such as regulation of apoptosis and cytokine production after *Pseudomonas* infection of airway epithelial cells. Lipid rafts are important for the regulation of NF-KB and the production of inflammatory cytokines after *Pseudomonas* infection. However, it also appears that lipid rafts may play a key role as a downregulator of certain chemokines including IL-1B in order to limit the inflammatory response.

The complex role of lipid rafts in the pathogenesis of *Pseudomonas* infections is just beginning to be understood but the mechanism of these diverse responses may be due to the sequestration of signaling molecules within rafts that determine their function. Another example of lipid raft-mediated regulation of cell signaling cascades and cytokine production has been revealed in studies of CD45 on T cells. CD45 is a cell surface molecule that is recruited to lipid rafts after T cell activation. Once localized to lipid rafts CD45 inhibits IL-2 production, whereas raft excluded CD45 is able to induce IL-2 production [58]. This compartmentalization of signaling receptors is increasing being recognized as a mechanism to regulate various signal transduction pathways. It was initially recognized that signal transduction pathways could be turned off by internalization of cell surface receptors and degradation within lysosomes [59,60]. More recently, it has been recognized that cell surface receptors can move in and out of lipid raft domains as a dynamic means of regulating their activity [61]. The pathogenesis of *Pseudomonas* shows that bacteria appear to have evolved means of altering these lipid raft-dependent cell signaling pathways to not only to increase bacterial internalization but also to modify the host inflammatory response. Alterations in host cell signaling pathways likely play a critical role in the pathogenesis of *Pseudomonas* as well as numerous other bacterial pathogens.

A large number of patients develop *Pseudomonas* colonization of the upper airways; however, only a small percentage of these patients develop clinically significant *Pseudomonas* pneumonia. The establishment of pneumonia is usually dependent on dissemination of *Pseudomonas* respiratory infections to the alveolar space [52,62,63]. The alveolar epithelium is the largest host epithelial surface exposed to the external environment with an area roughly equal to the size of a tennis court. Approximately 95% of that surface area is lined by specialized type I pneumocytes [52]. Type I pneumocytes are elongated, epithelial cells that perform gas exchange within the lung and are also constantly exposed to the external environment and bacterial pathogens. The cell membrane of type I pneumocytes has a high concentration of specialized lipid rafts and caveolae which occupy nearly 70% of the plasma membrane [64,65]. Invasion of type I pneumocytes would protect *Pseudomonas* from phagocytosis by alveolar macrophages and offer a protected environment for replication. *Pseudomonas* invasion of type I cells may facilitate dissemination throughout the host due to the single cell thickness of the alveolar epithelium. *Pseudomonas* invades type I pneumocytes during the pathogenesis of pneumonia both in vitro and in vivo. *Pseudomonas* invasion of type I-like cells is specifically inhibited by low doses of nystatin, filipin, and methyl-\(\beta\)-cyclodextrin. Intracellular *Pseudomonas* within alveolar epithelial cells is located within vacuolar membranes that are enriched in lipid raft components including caveolin-1 and -2 [66].

*Pseudomonas* invasion of alveolar epithelial cells was significantly decreased after RNAi downregulation of both caveolin-1 and caveolin-2 expression [66]. Since caveolin-1 is required for caveolin-2 expression, caveolin-1-deficient cells are also deficient in caveolin-2 [37,66]. Therefore, it has been speculated that caveolin-2 may play a novel, critical role in regulating caveolae mediated endocytosis. The mechanism by which caveolin-2 may influence the endocytic pathway is just beginning to be understood. Changes in caveolin protein expression may alter the membrane fluidity or alternatively caveolin expression may change host cell signaling pathways in order to influence endocytosis. *Pseudomonas* invasion is inhibited by the tyrosine kinase inhibitor, genistein, and increased after treatment with the phosphatase inhibitor, okadaic acid [66]. *Pseudomonas* infection of alveolar epithelial cells leads to increased tyrosine phosphorylation of caveolin-2. In addition, the changes in invasion after treatment with these drugs correlate with the changes in tyrosine phosphorylation of caveolin-2 [66]. *Pseudomonas* has the ability to invade both airway and alveolar epithelial cells via caveolin-enriched lipid raft and manipulate host cell signaling pathways. Thus, clustered lipid rafts appear to play a complex role in this evolutionary battle between the pathogen and the host defenses within the lung.

### 2.3. *Chlamydia trachomatis*

*Chlamydia* is an obligate intracellular bacterial pathogen that is believed to exploit lipid rafts in order to invade both phagocytic and non-phagocytic cells [67]. *Chlamydia* species are a common bacterial cause of sexually transmitted diseases as well as pneumonia. Similar to *E. coli* and *Pseudomonas*, disruption of lipid rafts with methyl-\(\beta\)-cyclodextrin, nystatin, or filipin inhibits invasion of *Chlamydia* species utilizing lipid rafts for entry [67]. The invasion of *C. trachomatis*, *pneumoniae* and *C. psittaci* have been shown to be inhibited by
removal of cholesterol and therefore they co-opt clustered lipid rafts as mechanisms of invasion [67]. However, not all species of Chlamydia are identical. Although C. trachomatis serovar D invasion in dependent on membrane cholesterol, serovars A and C are not inhibited by disruption of lipid rafts. Alternatively, these serovars are believed to enter cells via clathrin-coated pit-mediated endocytosis [67]. Finally, the mechanism of invasion used by serovars E and K is still controversial as opposite conclusions have been reached by separate investigators [67,68]. The different routes of entry are possibly due to the different cell surface molecules that serve as receptors for different serovars and whether or not they are located within lipid rafts on the plasma membrane of host cells. In addition, the route of invasion may differ based on the cell type or growth phase of the bacterial pathogen. Further evidence of the role of clustered lipid rafts in the pathogenesis of Chlamydia infections has been provided by confocal microscopy analysis of infected cells in vitro. Intracellular Chlamydia including C. pneumoniae and trachomatis co-localize with caveolin proteins. While caveolin-1 and -2 are both co-localized with C. pneumoniae and C. trachomatis serovars E, F, and K, only caveolin-2 was associated with C. trachomatis serovars A, B, C. In addition, caveolin-2 specifically accumulates with the bacterial pathogen at the inclusion membrane [69,70]. The microscopy studies provide further evidence that different C. trachomatis serovars have distinct mechanisms of invasion. Caveolin-1 does not appear to be required for Chlamydia uptake since invasion of FRT cells that lack caveolin-1 expression was not different than caveolin-1 expressing cells. Regardless of the expression of caveolin-1, the invasion of these strains is inhibited by disruption of lipid rafts [67]. These studies provide further evidence for the importance of caveolin-2, independent of caveolin-1 in the uptake of bacterial pathogens. Unlike E. coli and Pseudomonas, strains of Chlamydia are capable of actively modifying the composition of their intracellular compartments to promote intracellular growth [67]. Although each of these pathogens has evolved unique mechanisms of survival within host cells, their pathogenesis all require the integrity of clustered lipid rafts to survive.

2.4. Campylobacter jejuni

Campylobacter jejuni, one of the leading causes of diarrhea, is required to invade intestinal epithelial cells in order to cause clinical symptoms. It has long been recognized that campylobacter can invade intestinal epithelial cells and is viable within intracellular vacuoles [71]. While the receptor on epithelial cells for Campylobacter has not been clearly identified, lipid rafts are required for the entry of C. jejuni into the enterocyte-like cell line, Caco-2 [72]. Pre-treatment with filipin or cholera toxin significantly decreased the number of intracellular bacteria after campylobacter infection in vitro [72]. In addition, Campylobacter interaction with lipid rafts also affects host cell signaling pathways. Staurosporine, a non-specific kinase inhibitor, caused a dramatic reduction in the number of intracellular bacteria. Pre-treatment with the tyrosine kinase inhibitor, genistein, inhibited Campylobacter uptake but had no effect on the uptake of salmonella which utilizes a mechanism often defined as macro-pinocytosis to achieve entry into host cells. Finally, Campylobacter uptake was inhibited by wortmannin, a PI-3 kinase inhibitor [72]. Although not yet completely understood, these observations imply that Campylobacter are capable of co-opting clustered lipid rafts in order to achieve entry into host cells and the mechanism involves tyrosine phosphorylation of certain host cell substrates.

2.5. Brucella abortus

Brucella are gram-negative facultative intracellular bacteria which cause Brucellosis or Mediterranean Fever which is the most frequent human zoonosis, most prevalent in the Middle East and India [73]. Clinical manifestations of Brucellosis include fever, arthritis, endocarditis, and meningitis. A key event in the pathogenesis of Brucellosis is infection of macrophages and survival within membrane bound intracellular organelles. Brucella is an intracellular pathogen and one of the main virulence factors is its ability to invade macrophages via lipid rafts and replicate intracellularly. Brucella invades macrophages via a membrane ruffling and macropinosomes which selectively express lipid raft associated markers including GM1 and cholesterol [74]. After internalization, Brucella then inhibits phagosome–lysosome fusion and transits through a novel intracellular compartment [75]. Disruption of lipid rafts with methyl-β-cyclodextrin or filipin inhibits the lipid raft-mediated entry and survival of Brucella [75]. In addition, different species of Brucella have different means of invading host macrophages [76,77]. Brucella species express both rough and smooth variants of lipopolysaccharide (LPS) and the expression of the O side chain is recognized as a major virulence factor and an important determinant of bacterial entry. Smooth variants of LPS express the O side chain and avoid lysosomal fusion; however, rough mutants fail to express the O side chain and do not enter via lipid rafts [78,79]. Brucella is also known to contain a type IV secretion system which is important for bacterial invasion [80]. Recent work has shown that establishment of an intracellular Brucella infection is regulated by the activities of the type IV secretion system. Heat shock protein 60 (Hsp60) is secreted by Brucella and binds to the cellular prion protein prior to invasion. The cellular prion protein is incorporated into intracellular organelles that contain Brucella and deficiencies in the cellular prion protein prevented internalization and intracellular survival [77,81]. Brucella provides another unique and fascinating example how pathogens have learned to use host lipid rafts in order to gain a survival advantage and cause clinical manifestations of disease.

2.6. Mycobacteria

Mycobacteria are another intracellular pathogen that invade and survive within macrophages. Mycobacteria are successful in avoiding host defenses by resisting delivery to lysosomes and replicating within the mycobacterial phagosome following
entry into macrophages [82]. They are able to survive due to the active recruitment of a tryptophane-aspartate containing coat protein (TACO/Coronin 1) into the host cell membrane encasing the bacteria [83,84], TACO/Coronin 1 prevents fusion of the mycobacterial phagosome with the lysosome. Cholesterol is required for both the uptake of mycobacteria and for the association of TACO/Coronin 1 with the phagosomal membrane [83,84]. When murine macrophages were infected with Mycobacterium bovis, cholesterol accumulates at the site of bacterial entry and around intracellular mycobacteria. Depletion of cholesterol with lovastatin and methyl-β-cyclodextrin reduced Mycobacterium bovis and M. tuberculosis uptake by nearly 90% [84]. These agents contributed to the solubilization of TACO/Coronin 1 and disengagement from the phagosomal membrane. In the case of Mycobacterium avium, disruptors of lipid rafts inhibit bacterial infection of J774 murine cells by blocking the recruitment and formation of lipid rafts in areas of the plasma membrane in direct contact with bacteria [85]. Complement receptor 3 (CR3) is one of the receptors for mycobacteria that has been implicated in the lipid raft-dependent uptake [86]. CR3 is associated with GPI anchored proteins and is preferentially localized to lipid rafts. Antibodies against CR3 inhibit uptake of mycobacteria, L. casei, and zymosan. However, disruption of cholesterol only inhibited mycobacterial uptake and had no effect on the uptake of L. casei and zymosan [86]. Disruption of cholesterol redistributes CR3 to non-lipid raft regions of the plasma membrane [86]. Therefore, disruption of cholesterol does not inhibit CR3 function, but rather inhibits the complex interaction with CR3, GPI anchored proteins and cholesterol which is essential for the endocytic process. A number of mycobacterial species have been added to the growing list of pathogens who have been recognized to utilize lipid raft domains for invasion the invasion and survival within host cells.

3. Conclusion

Caveolae and lipid rafts play a number of important roles in normal cellular functions including cholesterol metabolism, cell signaling, and endocytosis [6,61]. Over the last several years, these microdomains have been recognized to play a role in the uptake of many intracellular pathogens including viruses, parasites, and bacteria [1]. The list of bacteria that depend on these cholesterol enriched domains for intracellular survival includes E. coli [38,46], P. aeruginosa [55,66], Chlamydia [67], Brucella [74,76], and mycobacteria [84,86]. Despite the many differences in the pathogenesis of these different infections, they all appear to utilize clustered lipid rafts in order to achieve entry and survival within host cells [5,15]. Each of these bacterial pathogens has significantly different mechanisms of pathogenesis and diverse clinical presentations, yet they share a very basic common mechanism of invasion. The evolutionary advantage provided by co-opting lipid raft-mediated endocytosis played an important role development of each of these unique pathogens. Bacteria have evolved mechanisms of co-opting clustered lipid rafts to invade a wide range of phagocytic and non-phagocytic cells. Once intracellular, these pathogens are able to avoid acidic lysosomes and intracellular degradation [3]. Caveolin-1 and -2 are two examples of key lipid raft associated proteins that have recently been implicated to play a key role in regulating this novel pathway of pathogen invasion [46,66]. The recent description of the caveolin-1- and -2-deficient mice [30,31] will hopefully allow an in vivo examination of the roles these proteins may play in the pathogenesis of a wide range of bacterial infections. This mechanism of invasion may allow replication within the protected intracellular environment and facilitate dissemination within the host. On the other hand, some investigators have implicated lipid raft-dependent endocytosis as a key component of the host defense against bacterial infection. The ability to internalize pathogens via clustered lipid rafts may signal apoptosis and allow the host to eliminate infected cells [55]. In addition to co-opting lipid raft-mediated endocytosis, pathogens can alter lipid raft associated signaling cascades. Lipid rafts cluster key signaling molecules and have been shown to regulate key components of the host defense against infections including inflammatory cytokine production [55,87]. The complex role lipid rafts play in these different aspects of bacterial pathogenesis is just beginning to be understood (Fig. 1). The list of pathogens that depend on clustered lipid rafts for invasion and intracellular survival will likely continue to grow. By studying the role of lipid rafts in bacterial pathogenesis, we may be able to get a better understanding of the disease mechanisms and hopefully design more effective treatment strategies. In addition, these pathogens allow us to better understand the natural conserved role that lipid raft-mediated endocytosis plays in cellular homeostasis.

References


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