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Review article

Manipulation of host cholesterol by *Helicobacter pylori* for their beneficial ecological niche

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ABSTRACT

Lipid rafts are mainly composed of phospholipids, sphingolipids, and cholesterol and dispersed in the cell membrane; these dynamic structures amplify signaling cues into the cells. A number of pathogens and virulence factors are found to favorably interact with lipid rafts as a major route to enter cells, followed by pathogenic effects. *Helicobacter pylori*, a gastric pathogen, subtly utilizes lipid rafts for its persistent inhabitation. In this review, we outline the underlying molecular mechanisms of the orchestration between membrane cholesterol and *H. pylori* as its infectious strategy. Understanding the particular niches for *H. pylori*-host equilibrium may provide novel approaches for the potential therapy of *H. pylori* infection.

1. Introduction

Helicobacter pylori, a spiral Gram-negative microaerophilic bacterium, colonizes in the stomach and infects approximately half of the human population in the world [1,2]. Persistent infection by *H. pylori* is associated with several clinical outcomes, including gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma [3,4]. *H. pylori* infection in the gastric mucosa is thought to be crucial in inducing stomach inflammation. *H. pylori* can evade host immune responses by utilizing its particular strategies to manipulate immune cells in the harsh environment of the stomach [5–8]. Additionally, *H. pylori* can penetrate across the mucosal layer, which may enable the bacteria to survive in the gastric epithelial cells [9,10]. Numerous reports have focused on the identification of virulence factors involved in pathogenesis and the underlying mechanisms that lead to different clinical sequelae in a specific host niche [11–14]. Interestingly, mounting evidence suggested that *H. pylori* exploits cholesterol-rich microdomains (also called lipid rafts) for the internalization of cells as many other pathogens. This review focuses on the role of lipid rafts in the initial step of *H. pylori* infection of host cells as well as how the bacteria manipulates this

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particular region for their own benefits and induction of pathogenesis.

2. Specialized membrane microdomains: lipid rafts

2.1. Structure and composition of lipid rafts

The major composition of lipid rafts includes cholesterol, sphingolipids, and phospholipids, which interact tightly and create rigid microdomains in the cell membrane [15]. The structure of lipid rafts is known to remain stabilized in cold nonionic detergents such as Triton X-100 [16]. After the treatment of the membrane with cold Triton X-100, insoluble components that were considered to be in the lipid rafts, including lipids and proteins, remain in the composition of detergent-resistant membrane (DRM), also called detergent-insoluble glycolipid-enriched membrane (DIG) [15]. Notably, the lipid rafts contained not only cholesterol and phospholipids, but also a significant level of glycosylphosphatidylinositol anchored proteins (GPI-APs), double-acylated proteins, and palmitoylated proteins [17,18]. It is thought that cholesterol exists in intact cell membranes between raft and nonraft regions. In the rafts, cholesterol serves as a spacer for sphingolipids and functions as a dynamic glue that keeps the lipids and proteins assembly tightly [19]. Several lipid rafts disruption agents, including methyl-β-cyclodextrin (MβCD), filipin, lovastatin, and nystatin, have been extensively employed in the investigation of their particular functions and compositions [19]. After the depletion of membrane cholesterol by MβCD or filipin, the raft-associated proteins and lipids can be dissociated and, rendering the structure nonfunctional [20,21].

2.2. Involvement of lipid rafts in microbial infection

Lipid rafts are not only a dynamic structure on the cell membrane, but also provide an amplified signaling for the activation of the cells [22]. Those are also known to invaginate the cell membrane and lead to endocytosis mediated through a different clathrin-mediated endocytosis [23]. Several studies have demonstrated that lipid rafts might serve as platforms for entry portals of pathogens, including bacteria [24–32], viruses [33–37], as well as prions [38]. There are two possible benefits for pathogen entry through rafts: one is the prevention of intracellular degradation, and the other is the triggering of signaling that causes membrane fission and cytoskeleton rearrangement, which are both required for bacterial infection [39]. Therefore, it can be suggested that pathogens may favorably interact with lipid rafts, which are potential gateways to enter the host cells.

2.3. Lipid rafts provide a route for bacterial components to interact with host cells

One of the popular examples of lipid rafts as bacterial entry portals is *Shigella flexneri*, which harbors a type III secretion system (TTSS) for the induction of pathogenesis in the host cells. When *S. flexneri* attaches to the host cells, the TTSS is activated and the bacterial effectors are directly injected into the cytoplasm [40]. The machinery of bacterial effectors' delivery into host cells is dependent on the interaction of cholesterol with TTSS [41]. The bacterial effector, IpaB, is found to interact with the raft-associated CD44 within the specialized membrane microdomains [25]. Similar to *S. flexneri*, *Salmonella enterica* employed a TTSS to invade into the host cells. Likewise, cholesterol is not only required for entry of *S. enterica*, but also for providing cholesterol-rich vacuoles for bacterial multiplication and survival inside the cells [32]. Type 1 fimbriated *Escherichia coli* is also found associated with caveolae and raft components that may facilitate bacterial internalization and enable bacteria to survive intracellularly [26]. Additionally, some bacteria capitalize on an amplified host inflammatory response by co-opting raft-associated signaling. For instance, *Pseudomonas aeruginosa,* those cholesterol-rich microdomains are served as platforms for bacterial attack, whereas also for host counterattack [27].

It has been reported that nonintracellular bacteria also hijack host membrane rafts for delivery of their toxins [39]. The most important example is the cholera toxin subunit B from *Vibrio cholera* bound to ganglioside GM1, which is localized in the raft microdomains [42]. The anthrax toxin produced by *Bacillus anthracis* is also targeted to its receptor via a lipid raft-mediated clathrin-dependent process [43]. Moreover, a bacterial membrane-associated protein—cytolethal distending toxin (Cdt)—secreted by *Actinobacillus actinomycetemcomitans*, *Campylobacter jejuni*, or *Haemophilus ducreyi* is found interacted with lipid rafts, leading to the cytopathic effect by its genotoxicity [44–46]. Overall, the interaction of toxins with lipid rafts may play an important role in the toxin delivery inside cells and magnify the signaling for their pathogenesis (Table 1) [30, 42–55].

3. *H. pylori* virulence factors

H. pylori contains a set of virulence factors that enables it to survive, multiply, escape from immune surveillance, and eventually lead to persistent infection in a particular niche of the host. Although gastric mucosa is well protected against other bacterial infection, *H. pylori* is highly adapted to its

Table 1 – Bacterial toxins that interact with cholesterol-rich microdomains.

Pathogen	Bacterial toxin	References
Actinobacillus actinomycetemcomitans	Cytolethal-distending toxin	[44]
Aeromonas hydrophila	Aerolysin	[47]
Bacillus anthracis	Anthrax toxin	[43]
Campylobacter jejuni	Cytolethal-distending toxin	[46]
Haemophilus ducreyi	Cytolethal-distending toxin	[45]
Helicobacter pylori	Vacuolating cytotoxin A	[48–52]
	Cytotoxin-associated gene A	[30,53]
Listeria monocytogenes	Listeriolysin O	[54]
Vibrio cholera	Cholera toxin	[42]
	Cytolysin	[55]

ecological niche. These approach support the colonization and persistence of *H. pylori* in the gastric mucus, including polar flagella, urease, adhesins, and two major virulence factors: vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA) [56]. Previously, on the basis of the expression of CagA and VacA, *H. pylori* clinical isolates are grouped into two major types: type I strains harbor VacA and CagA, whereas type II bacteria do not express VacA or CagA [57]. Type I strains are closely associated with peptic ulcers and thus considered to be more virulent [58]. In addition to VacA and CagA, an important study by Wunder et al. revealed that the *H. pylori* enzyme, cholesterol-α-glucosyltransferase, which is responsible for cholesterol glucosylation in macrophages, is thought to have modulated the innate immunity [5].

3.1. *VacA hijacks lipid rafts*

VacA, one of the major virulence factors of *H. pylori*, is secreted from the bacteria and found in the culture supernatant [59], but a large portion remains on the bacterial surface [60]. After *H. pylori* colonizes the cells, the bacterial surface-contacted VacA directly transfers from the bacteria to the host cells, followed by uptake and intoxication. VacA is also reported to inhibit T-cell proliferation, suggesting that *H. pylori* might evade the adaptive immune response of the host [11]. Apart from its cytotoxicity, VacA is first reported to exploit cholesterol-rich microdomains for its assembly on the cell membrane and intracellular delivery [48]. Several reports reveal that depletion of membrane cholesterol significantly reduces the assembly and entry of VacA into the target cells [48–50]. We and Gauthier et al. found that VacA exploits rafts via the endocytic pathway of GPI-APs [51,61]. The binding of VacA to lipid rafts is then observed by atomic force microscopy at pH 4.0 [62]. However, this effect did not occur at pH 7.6, suggesting that cholesterol is essential for VacA assembly in lipid rafts, preferring an acid environment.

Although the receptor-dependent translocation of VacA to lipid rafts is found to be critical for signaling pathways, including p38 MAP kinase/ATF-2 activation and toxin-mediated vacuolation [63], the specificity of the VacA-raft interactions remained unclear at that time. The evidence from our previous study indicated that a GPI-AP, fasciclin I, is required for the internalization of VacA in epithelial cells [51]. However, this report shows that the binding of VacA to lipid rafts is not affected by the presence of fasciclin I. Further, Gupta et al. found that sphingomyelin is important for VacA association to lipid rafts and for VacA-mediated vacuolation [52]. This trend occurs not only in gastric epithelial cells of AGS and AZ-521, but also in other mammalian cells, including CHO-K1, Vero, and HeLa cells, suggesting that VacA exploits sphingomyelin as a specific receptor in the cholesterol-rich microdomains.

Little evidence suggests that *H. pylori* is a dominant intracellular pathogen. However, the infection models *in vitro* and *in vivo* show that *H. pylori* can survive and replicate in the intracellular niche [64–66]. Moreover, type I *H. pylori* (CagA+/CagA+) delays uptake by macrophages, followed by the formation of megasomes as a result of phagosome fusion [67]. Likewise, VacA-expressed *H. pylori* promotes the recruitment and retention of the tryptophan aspartate-containing coat protein (TACO) by phagosomes, thereby disrupting the phagosome maturation in monocytes and promoting enhanced survival of this bacteria [68]. Both the depletion of cholesterol and mutation of VacA show significantly reduced *H. pylori* internalization in gastric epithelial cells [30]. Additionally, the infection of *H. pylori* induces autophagosome formation not only in phagocytes but also in gastric epithelial cells [69]. This observation is in line with the result of cell treatment with VacA that induces autophagosome formation [70,71], and it is supported by a previous study, which demonstrated that the internalization of pathogen and stimulation of autophagy are cholesterol-sensitive [72]. Although the association of *H. pylori* exploitation of lipid rafts and triggering of autophagy has not been well investigated, the bacteria survives intracellularly and is protected from antimicrobial agent therapy [10,73].

3.2. *CagA exploits cholesterol-rich microdomains*

Another major virulence factor of *H. pylori* is the *cag* pathogenicity island, (*cag* PAI)-encoded type IV secretion system (TFSS), which mediates the translocation of CagA cytotoxin-associated gene (CagA) into host cells [74,75]. On injection, CagA is subsequently phosphorylate at one or more tyrosine phosphorylation in the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs and induces host cell pathogenesis, which includes a phenotype associated with cell scattering (the hummingbird phenotype) [75], induction of nuclear factor (NF)-κB activation and interleukin (IL)-8 secretion [76], and disruption of tight junctions [77]. Higashi et al. first indicated that the EPIYA motif is responsible for the association of CagA with cell membrane, presuming that the translocation of CagA occurs in membrane rafts [78]. Our study then demonstrates that the disruption of lipid rafts by the depletion of cholesterol attenuates CagA translocation, hummingbird phenotype, and IL-8 secretion, suggesting that the delivery of CagA into epithelial cells is mediated in a cholesterol-dependent manner [30]. Subsequently, the CagA C-terminal domain-containing EPIYA regions directly target the lipid rafts of gastric epithelial cells, which was further reported by our recent study; however, the number of EPIYA motifs does not interfere with the ability of CagA to bind to raft microdomains [79].

A recent study by Murata-Kamiya et al. demonstrates that the initial contact of *H. pylori* with cells induces phosphatidylserine externalization from the inner leaflet of the cell membrane to the outer leaflet, thus facilitating the translocation of CagA into the cytoplasm [53]. The translocated CagA is then tethered in the inner leaflet of the plasma membrane through the direct binding of phosphatidylserine. Moreover, CagA is shown to interact with partitioning-defective 1b (PAR1b)/microtubule affinity-regulating kinase 2 (MARK2), which contributes to the disruption of tight junctions [77]. The infection of cells with *H. pylori* recruits MARK2 from the cytoplasm to the plasma membrane, which localizes mainly in DRMs [80]. These results indicate that *H. pylori* CagA is able to delicately manipulate lipid rafts for its functioning in host cells and to establish a successful infection.

H. pylori peptidoglycan (PG) has previously shown to be recognized by nucleotide-binding oligomerization domain protein (NOD1) and delivered by TFSS to host cells [81]. Kwok et al. reported that *H. pylori* CagL is presented in TFSS and

activated $\alpha_5\beta_1$ integrin, which triggers CagA translocation and Src kinase activation [82]. This interaction is then found to occur in lipid rafts, which requires the delivery of PG to NOD1 and activation of NF-κB-dependent responses to *H. pylori* [83]. All these findings suggest that *H. pylori* TFSS involves specialized $\alpha_5\beta_1$ integrin interaction in cholesterol-rich microdomains, which leads to intimate attachment and efficient delivery of the bacterial components to the host cells.

4. Modulation of cholesterol regulates immune responses by *H. pylori*

A previous report by Wunder et al. shows that *H. pylori* followed a cholesterol gradient and extracted the lipid from cytoplasmic membranes of epithelial cells for subsequent glucosylation [5]. A crucial enzyme, cholesterol-α-glucosyltransferase encoded by HP0421, is identified to be responsible for cholesterol glucosylation in macrophages, hence protecting *H. pylori* from phagocytosis and reducing the antigen-specific T-cell responses [5,84]. The exposure of human $CD4^+$ T cells to isogenic HP0421 mutant *H. pylori* results in the significant inhibition of T-cell proliferation [85]. We recently demonstrated that the glycosylated cholesterols synthesized by *H. pylori* partition in cholesterol-rich microdomains around host-pathogen contact sites alters membrane architecture. Further, HP0421-knockout *H. pylori* significantly lost the *cag* TFSS-associated activity [86].

Toll-like receptors (TLRs) are pattern-recognition receptors that recognize conserved microbial components [87]. Lipopolysaccharide (LPS) from *H. pylori* is reported to induce the formation of TLR2, TLR1, CD36, and CD11b/CD18 complexes in lipid rafts, leading to TLR2-mediated inflammatory responses in vascular endothelial cells [88]. In contrast, evidence supports the possibility that *H. pylori* LPS-induced signal transduction in gastric epithelial cells is mediated through TLR4 [89]. Another *H. pylori* virulence factor, HP0175, is found be able to transactivate epidermal growth factor receptor through TLR4, which occurred in lipid rafts [90]. Our recent finding indicates that ceramide and TLR4 are mobilized to membrane rafts and involved in the regulation of *H. pylori*-induced inflammatory responses [91]. These lines of evidence support the hypothesis that that manipulation of cholesterol by *H. pylori* during the infection, which facilitate the bacteria to colonize the unique niche in the host, evade immune surveillance, stimulate inflammatory responses, and eventually lead to chronic diseases in the host (Fig. 1).

5. Conclusions and perspectives

It is now evident that several virulence factors from *H. pylori* are able to exploit or modulate cholesterol to gain a foothold in the host niche. The molecules distributed in the cholesterol-rich microdomains sense and respond to *H. pylori* via an orchestrated manner during persistent infection, which together play a role in disease progression. It is believed that *H. pylori* exploits cholesterol for two purposes. First, the lipid rafts efficiently serve as a platform required for the delivery of bacterial virulence factors. Second, the lipid rafts provide a gateway for

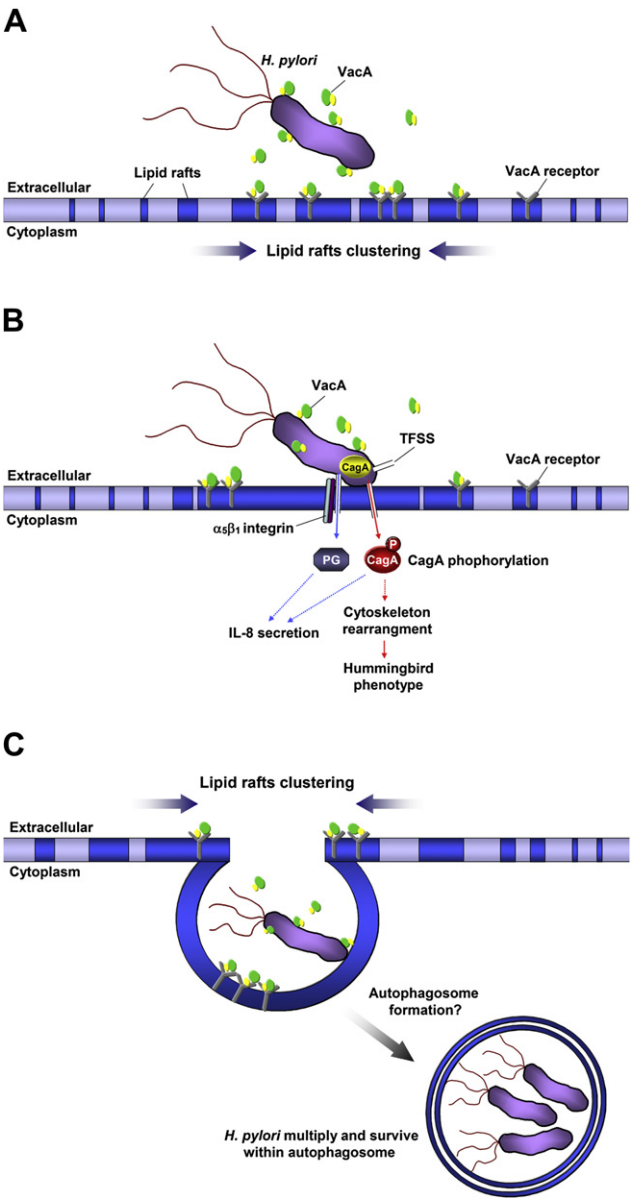

Fig. 1 – Model depicting *Helicobacter pylori* manipulating host cholesterol through an infectious strategy. (A) VacA secreted by *H. pylori* binds to receptors, which are localized in the cholesterol-rich microdomains, and facilitates rafts which coalescence into the sites of bacteria infection. (B) The clustering rafts initiate TFSS to inject CagA and PG into the cytoplasm and induce downstream signaling events. (C) The raft-associated membrane may extend to form autophagosomes, which provide compartments for *H. pylori* to survive intracellularly. CagA = cytotoxin-associated gene A; IL-8 = interleukin-8; PG = peptidoglycan; TFSS = type IV secretion system; VacA = vacuolating cytotoxin A.

H. pylori infection inside the host cells and multiply in a particular niche for its long-term persistent infection. While both the hypotheses posit the pathogenesis by *H. pylori*, supporting that this bacteria manages the dynamics of the membrane rafts for its benefits. The depletion of cholesterol is

found to be successful in anti-HIV activity, particularly in decreasing viral replication and production [92]; these results shed light on the new therapeutic approach: inhibition of cholesterol-enriched binding sites for microbial infection. Therefore, it is worthy to develop therapeutic agents that inhibit lipid rafts, which may lead to the failure of *H. pylori* infection in the initial stage. In parallel, understanding the molecular mechanism for pathogen-host interaction may provide an insight into the development of novel strategies that target lipid rafts to control the infection of these pathogens.

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