Review Article

Role of lipid rafts in persistent *Helicobacter pylori* infection: a narrative review

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Background and Objective: The distribution of components in the cell membrane is not uniform, but is organized into specific functional microdomains, known as "lipid rafts". These lipid rafts consist of cholesterol, sphingolipids, and various proteins. Studies have shown that lipid rafts contain multiple proteins that are closely related to signal transduction and immune response. Furthermore, lipid rafts are the sites where a variety of pathogens invade the cells, and are associated with the persistent infection of some pathogens, especially *Helicobacter pylori* (Hp). We are going to explore a new method to treat Hp by discussing the important role of lipid rafts in Hp persistent infection.

Methods: Papers on lipid rafts were retrieved to analyze the evolution of the definition of lipid raft, research techniques, and studies on the correlation of lipid rafts with pathogens infecting host cells.

Key Content and Findings: Hp uses cholesterol-α-glucosyltransferase (CGT) to extract cholesterol from the lipid rafts of host cell membrane and destroys the integrity of the lipid rafts, which contributes to its immune escape; Using drugs to inhibit the destruction of lipid rafts by CGT can inhibit the growth of Hp and help the body clear Hp.

Conclusions: Lipid rafts are key to persistent Hp infection, and a new field of research on pathogen-host cell interactions and signal transduction. Researches on lipid rafts may promote a new breakthrough in the field of treatment of Hp.

Keywords: Lipid raft; cholesterol; immunity; *Helicobacter pylori* (Hp); persistent infection

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Introduction

Cell membrane is an elastic, fluid structure composed of a phospholipid bilayer (skeleton) and multiple other substances that separates the intracellular and extracellular environments. It carries out specific functions such as barrier protection, selective permeability, endocytosis/secretion, cell-cell recognition, and signal transduction, to name a few. It is also the place where some enzymatic reactions take place (1). The substances enclosed by the cell membrane are not always homogenously distributed, but are compartmentalized into a batch of special functional regions formed by high aggregation of certain substances that are often present at low levels outside these special domains (2). Lipid rafts are one of such functional microdomains that contain cholesterol, sphingolipids, and proteins (3), with

Table 1 The search terms used

("Membrane Microdomains"[Mesh]) AND "Helicobacter pylori"[Mesh]
("Helicobacter pylori"[Mesh]) AND "Infections"[Mesh]
"Membrane Microdomains"[Mesh]
"Helicobacter pylori"[Mesh]
("Helicobacter pylori"[Mesh]) AND "Disease Eradication"[Mesh]
("Membrane Microdomains"[Mesh]) AND "Infections"[Mesh]
("Membrane Microdomains"[Mesh]) AND "Inflammation"[Mesh]
("Membrane Microdomains"[Mesh]) AND "Therapeutics"[Mesh]
("Helicobacter pylori"[Mesh]) AND "Cholesterol"[Mesh]
("Helicobacter pylori"[Mesh]) AND "Neoplasms"[Mesh]

many tumor- (4) and immunity-associated (5) molecules. Lipid rafts are exploited by intracellular pathogens as a gateway (6,7) to invade the cells, making it a new hot spot in the studies on host cells and pathogen infections. At present, researchers regard lipid rafts potential therapeutic targets for some diseases (8) in the search for the mechanisms and new therapies of tumorigenesis or persistent infection.

Helicobacter pylori (Hp) is a Gram-negative, health-threatening bacterium that is estimated to have infected more than 50% of the global population (9). In most cases, there are no obvious clinical symptoms after infection, and only a small number of infected people develop gastritis, gastric ulcers, or other diseases. In severe cases, Hp can lead to stomach cancer. It is difficult to eradicate due to its ability to evade the immune system (10) via a variety of mechanisms. Thus, Hp infections are often long-lived. Recent study has revealed that persistent Hp infection is closely associated with the development of gastric cancer, so eradication of Hp can lower the risk of gastric cancer (11). In 2012, the World Health Organization (WHO) classified Hp (infection) as a class 1 carcinogen.

The clinical treatment of Hp patients has long been dominated by antibiotics (12), but with the continuous expansion of Hp eradication indications and the increasing rate of drug resistance (13), it is necessary to discover novel treatment strategies. Research on vaccines and non-antibiotic drugs for the prevention and treatment of Hp infection is currently the focus of the medical field. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1000/rc).

Methods

Relevant studies published over the last 20 years were identified via a PubMed search using different combinations of the following search terms: "Helicobacter pylori", "lipid raft/membrane microdomain", "infection", "therapeutic/treatment", "cholesterol". Most of the search formulas we use are shown in *Table 1*. Additional papers were identified by reviewing reference lists of relevant publications. Publications with relative low credibility and non-English publications were excluded. Data were extracted based on their relevance to the topic instead of implementing a systematic approach to paper selection. More details of the method are shown in *Table 2*.

What is the nature of lipid rafts?

In 1973, Yu *et al.* used the non-ionic detergent Triton X-100 to treat cells, and discovered that it was difficult to solubilize some components in the lysate with the detergent (14). In 1997, Simons *et al.* put forward the concept of "lipid raft" for special structures on the cell membrane, namely, clustering of sphingolipids and cholesterol as a lipid "raft" to which some special proteins can be anchored. This "lipid raft" can form a liquid-ordered (Lo) functional area on the liquid-disordered (Ld) cell membrane for the cell to carry out specific physiological activities (3). Subsequently, an interaction of preferential aggregation between some of the lipids was observed on the biomimetic model (biomembrane), resulting in liquid-liquid phase separation (15), a phenomenon that further supports the "lipid raft" theory. In 2006, a conference updated the definition of lipid raft: lipid rafts are tiny (10–200 nm), heterogeneous, dynamic (lateral movement across the membrane, dynamic polymerization-dissociation), cholesterol- and sphingolipid-rich membrane nanodomains that compartmentalize cellular life activities; they can also form larger platforms through protein-protein interactions and protein-lipid interactions (>300 nm) (16). The microscopic scale of 10–200 nm is beyond the resolution of an optical microscope, and this has delayed the conduction of experiments for direct observation of lipid rafts. With further studies, there is now a tendency, based on the above definition, to regard lipid raft as a transient, relatively ordered membrane nanodomain (17), which is in a dynamic process of formation and dissolution (18) rather than a long-term stable inherent structure. This means

Table 2 The search strategy summary

Items	Specification
Date of search (specified to date, month and year)	2021/12/07–2022/01/04
Databases and other sources searched	PubMed
Search terms used (including MeSH and free text search terms and filters)	See *Table 1* for details
Timeframe	1973–2021
Inclusion and exclusion criteria (study type, language restrictions etc.)	Inclusion criteria: research articles and reviews in English about themes such as *Helicobacter pylori* and lipid raft. Exclusion criteria: some papers which we considered with low reliability
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Renjie Liu conducted the selection, all authors attended a meeting to discuss the literature selection and obtained the consensus
Any additional considerations, if applicable	Some papers were identified by reviewing reference lists of relevant publications

that except the lipid composition is roughly the same, there are different proteins in lipid rafts of different cells, and the components on the same lipid raft also change over time.

In what ways are lipid rafts researched?

In the process of verifying the authenticity of this structure, scientists have constructed a wide range of model membranes and employed numerous research techniques to study the morphological and kinetic aspects of lipid rafts. Baumgart *et al.* [2007] demonstrated that the giant plasma membrane vesicles (GPMVs) induced on the plasma membranes of cultured mammalian cells can also be separated into micron-scale fluid phase domains, suggestive of a non-ideal, non-randomized distribution (19) of membrane components in living cells (19), further corroborating the existence of lipid rafts, and providing a classical tool to understand the formation and kinetics of lipid rafts. Chiantia *et al.* [2006] used atomic force microscopy (AFM) and dual-focus fluorescence correlation spectroscopy (2fFCS) (20) and Štefl *et al.* [2012] used 2fFCS and cross-linking agents (21) to study the lipid distribution in dioleoyl phosphatidylcholine/sphingomyelin/cholesterol (DOPC/SM/cholesterol) model membranes. These studies have clearly confirmed the fact that some components spontaneously aggregate in cell membranes and liquid-liquid phase separation in the natural state, making important contributions to improving lipid raft-related theories. Recently, the development of novel techniques such as super-resolution observation technology has brought hope to direct observation of lipid rafts. Ando *et al.* [2015], for instance, studied the distribution of sphingolipids in lipid rafts on artificial monolayers with the help of a Raman microscope (22). Dumitru *et al.* [2018] observed the lateral structure of lipid membrane under AFM and a toxin derivative probe (23). Ryu *et al.* [2019] used epi-fluorescence microscopy (EFM) to confirm that cholesterol infiltration into sphingolipid-rich structural domain plays a vital role in membrane phase separation (24). These studies on the physical properties of lipid rafts have greatly promoted the development of the lipid raft theory.

The locating and tracing of the components on lipid rafts by tracers and fluorescent labeling techniques have greatly enriched and simplified the methods for studying lipid rafts. Researchers have noticed that the specific ligand recognized by the cholera toxin B subunit (CTB) is the ganglioside GM1 in the cell membrane (25), and GM1 is highly enriched in lipid rafts. Scientists have recently chosen CTB as a marker molecule to trace lipid rafts, and have carried out a lot of fruitful research (26-28). More and more new tracing techniques have been developed, such as fluorescently labeled gangliosides for lipid raft imaging synthesized by Konishi *et al.* in 2020 (29), and the strategy established by Shi *et al.* in 2021 for analyzing the glycan distribution in lipid rafts and beyond (30). These labeling and tracing techniques have greatly promoted the study of lipid raft structures and functions, and provided new ideas for the research of lipid raft components-

targeted drugs (31).

Lipid rafts and persistent Hp infection

Are there studies on both lipid rafts and infection of host cells with pathogens?

Lipid rafts are enriched with substances that are less common in other cell membrane regions, such as cholesterol, in addition to proteins with distinct functions. The proteins that have their own functions and the lipids that form liquid-liquid phase separation make the functions of lipid rafts complicated.

Early studies have shown that lipid raft components like cholesterol are of great significance to the entry and survival of influenza virus in the cell (32-34), and this finding continues to inspire many researchers, such as Ohkura *et al.*, who found that the binding of influenza A virus hemagglutinin and neuraminidase to lipid rafts promoted it's trafficking (35), and Verma *et al.*, who demonstrated that the lipid rafts of the host cell play a leading role in the binding and endocytosis of influenza A viruses (36). Lipid rafts also have a place in the currently hottest field of severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) research. For instance, Fantini *et al.* concluded that the effective action mechanism of hydroxychloroquine in combination with azithromycin for the treatment of SARS-CoV-2 is related to the sphingolipids GM1 on lipid rafts: because of a structural similarity between azithromycin and GM1 glycan part, azithromycin is able to competitively bind the ganglioside-binding domain of the SARS-CoV-2 spike protein; hydroxychloroquine, on the other hand, is able to saturate the virus attachment sites of ganglioside near the coronavirus receptor of host cell membrane (37). The combined use of these 2 drugs bidirectionally blocks the binding of the virus to the host cell for antiviral purposes (37). In addition, Gekara *et al.* showed that *Listeria monocytogenes* can induce the concentration of host cell lipid rafts via listeriolysin O (38). Fine-Coulson *et al.* also discovered that the induction of lipid raft aggregation during infection of type II alveolar epithelial cells could be caused by *Mycobacterium tuberculosis* (39). All of these studies suggest that lipid rafts are closely associated with pathogen-host interactions in several diseases, and studying the mechanism of action of lipid rafts in diseases is of great significance for the treatment of these diseases. Then, do lipid rafts play an important role in the persistence of Hp infection?

How do lipid rafts mediate signal transduction in host cells during persistent Hp infection?

Lipid rafts, as an important platform for signal transduction, are capable of recruiting a variety of inflammatory signaling-related receptors. Kwok *et al.* demonstrated that Hp injects cytotoxin-associated protein (CagA) and peptidoglycan into host cells via its type IV secretion system binding to integrin α5β1, so that nuclear factor-κB (NF-κB) is activated and proinflammatory cytokines such as interleukin 8 (IL-8) (40) will be produced, while α5β1 is localized on lipid rafts (41). In other words, Hp induces NF-κB-dependent inflammatory response in gastric epithelial cells through lipid rafts (*Figure 1A*) (42). Lin *et al.* pointed out that Hp can also promote the inflammation of gastric epithelial cells by activating the expression of high-mobility group box 1 (HMGB1) and recruiting the receptor for advanced glycosylation end-products (RAGE) to lipid rafts to activate the NF-κB/IL-8 signaling pathway (*Figure 1B*) (43). These findings have demonstrated that Hp promotes the inflammatory response of the body by recruiting inflammatory factors to lipid rafts in diverse ways. Blouin *et al.* also showed that proper assembly of the interferon-γ receptor (IFNGR) subunits IFNGR1 and IFNGR2 on lipid rafts is critical for IFNG/JAK/STAT signaling transduction, as shown in *Figure 1C* (44), meaning that lipid rafts are also important platform for IFNG signal transduction. Then, how does Hp evade the killing by these inflammatory responses and form persistent infection of the host cell?

Why does persistent Hp infection destruct lipid rafts?

Trampenau *et al.* found that Hp has a specific affinity for cholesterol in the process of culturing (45), and the large amount of cholesterol (3) enriched in lipid rafts seems to suggest a possible link between Hp and lipid rafts. Haque *et al.* discovered that the lipid composition of Hp contains an abundant number of characteristic cholesteryl glucosides (CGs) (46). The experiments by Testerman *et al.* confirmed that exogenous cholesterol is required for the growth of Hp during *in vitro* culturing (47), suggesting that Hp does not appear to possess an enzyme for cholesterol synthesis. So, where does the cholesterol required for the synthesis of CGs by Hp during *in vivo* infection come from? It must be that, in some way, Hp obtains a profuse amount of cholesterol for the synthesis of CGs from the host *in vivo*. However, the largest cholesterol reservoir in the

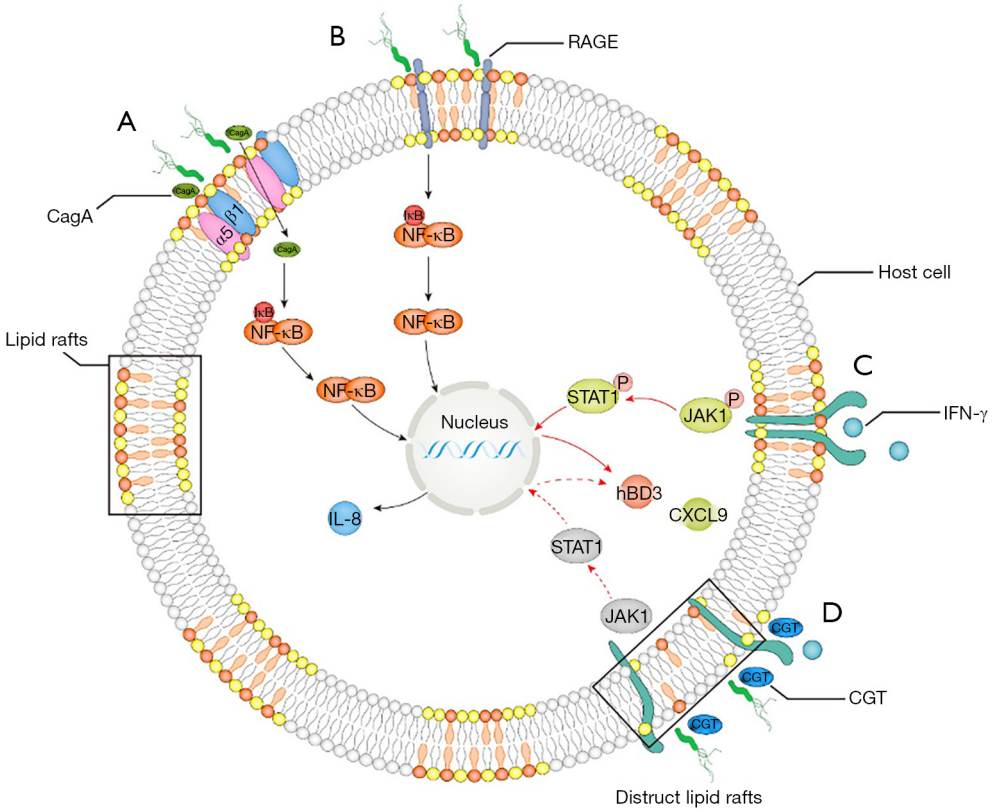

Figure 1 Signal transduction mediated by lipid rafts in persistent Hp infection. (A) Hp infects the host cell by binding to integrin α5β1 on lipid rafts and injects CagA into the host cell to activate the NF-κB/IL-8 signaling pathway and promote inflammation. (B) Hp recruits RAGE into the lipid raft to activate the NF-κB/IL-8 signaling pathway and promote inflammation. (C) The correct assembly of IFNGR subunits is dependent on the lipid raft integrity and essential for IFNG/JAK/STAT signal transduction. (D) Hp uses CGT to disrupt the structure of the lipid raft on the host cell membrane, making IFNGR unable to assemble properly, which in turn frustrates IFN-γ signaling, and helps Hp evade inflammatory response. RAGE, receptor for advanced glycosylation end-products; CagA, cytotoxin-associated protein; NF-κB, nuclear factor-κB; IL-8, interleukin 8; IFN-γ, interferon-γ; CGT, cholesterol-α-glucosyltransferase; Hp, *Helicobacter pylori*; IFNGR, interferon-γ receptor.

surrounding environment of Hp infection is the lipid rafts in the gastric epithelial cell membrane. This seems to imply the disruption of host cell lipid rafts by Hp.

In 2006, Wunder *et al.* identified *hp0421*, a gene encoding a cholesterol-α-glucosyltransferase (CGT) that can extract cholesterol from host cell lipid rafts, and elucidated that CGT plays the role of extracting cholesterol from the lipid rafts on the host cell membrane to synthesize CGs; they also found that Hp strains whose *hp0421* genes are knocked out are more readily ingested by macrophages, and the knockout strain stimulated T cell activations compared to the wild strain (48). Furthermore, mice challenged with the knockout strain were cleared of bacteria after a period of time compared with 2 controls, namely, wild-type strains, and strains of CGT gene-containing plasmids (48), suggesting that the function of CGT is important for the immune escape of Hp, perhaps because the extraction of cholesterol by CGT has impaired lipid rafts, and thus inhibited phagocytosis of phagocytes and T cell-dependent immune response.

Morey *et al.* found that the downstream JAK/STAT1 pathway failed to be activated by IFNG after prolonged infection while treating Hp-infected MKN45 cells with interferon-γ (IFN-γ) (49). The key factor CGT responsible for this effect is subsequently identified through experiments performed by knocking out the virulence genes of Hp strains one by one (49). The mechanistic pattern is shown in *Figure 1D*. As Blouin *et al.* had demonstrated the

importance of proper assembly of IFNGR on lipid rafts for IFNG/JAK/STAT signaling (44), Morey *et al.* then speculated: does Hp inhibit the inflammatory response of the host cell by disrupting the integrity of lipid rafts and thus affecting the correct assembly of IFNGR through CGT extraction of cholesterol from the lipid rafts on the host cell membrane (49)? To test this hypothesis, they treated the cell with methyl-β-cyclodextrin (capable of solubilizing the cholesterol in cell membranes), and found that JAK1/STAT1 phosphorylation levels were significantly reduced; at the same time, after protecting the integrity of cell membrane lipid rafts via a cholesterol coating prior to infection, the IFNG/JAK/STAT signals in the cells infected with wild-type Hp whose CGT gene is not knocked out can be normally transduced, and JAK1/STAT1 is normally phosphorylated to regulate downstream gene expression. Furthermore, by adding cholesterol to the culture system already infected with wild-type Hp, the impaired IFNG signaling is partially restored (49). These experiments have confirmed that Hp can weaken the lipid raft to block the IFNG signaling pathway, and thus achieve immune escape. Similarly, they found that Hp also inhibits the IFNB, IL-6, and IL-22 signal transduction of the receptors localized on lipid rafts, as well as the downstream human β-defensin 3 (HBD3) response, by disrupting lipid rafts (49). Cholesterol extraction by Hg CGT from the lipid raft helps to inhibit multiple inflammatory signaling pathways allows for continuous, localized Hp colonization. So, are other inflammatory signaling pathways such as NF-κB affected? Further investigation is required.

What can we do to repair the lipid rafts affected by Hp infection?

Morey *et al.* demonstrated that adding cholesterol to a co-culture system where the cells are infected with wild-type Hp and IFNG signaling is suppressed can up-regulate JAK1/STAT1 phosphorylation (49), implying that the addition of cholesterol repairs part of the lipid rafts, allows some IFNGR to assemble properly, and unblocks the IFNG/JAK/STAT inflammatory signaling pathway. Are other inflammatory signaling pathways affected? Does this phenomenon have practical significance for the control of Hp infection? Wunder *et al.* demonstrated that, by continually adding 2% cholesterol to the diets of Hp-infected mice, the bacterial load is reduced by more than 95% compared to cholesterol-free diets, and in the gastric tissue samples of the mice with reduced bacterial loads there is massive infiltration of neutrophils and lymphocytes, whereas no inflammatory infiltration was observed in the tissues cut from the stomachs of the uninfected mice that eat the food containing 2% cholesterol (48).

Kobayashi *et al.* added the cholesterol analog, cholestenone, to the co-culture system of Hp and gastric cancer cells, and found that it also restrains the synthesis of CGs in Hp (50). This cholesterol analogue differs from cholesterol in that the hydroxyl group on the third C atom of cholesterol that can be linked to glucosides under the influence of CGT is turned into a carbonyl group, making CGT unable to attach the glucoside. In contrast, 2 other cholesterol analogs, namely β-sitosterol and cholestyramine, that modify the carbon atoms at other positions, do not have anti-Hp activities (50). Moreover, during *in vitro* experiments, the cholestenone can efficiently resist Hp regardless of whether there is cholesterol in the system. This effect is enhanced with increasing concentrations of cholestenone (50). The addition of 0.5% cholestenone to the diet can greatly reduce the bacterial load in the gastric mucosa of C57BL/6 mice already infected with the Hp SS1 strain (50). More delightfully, cholestenone is also effective against clarithromycin-resistant Hp strain 2460 (50). This brings new hope to eradication therapy for Hp, which is now plagued by rising drug resistance rates.

Kawakubo *et al.* discovered that the mucous cells deep in gastric mucosa can secrete O-glycans with α1,4-N acetylglucosaminoglycan terminals. Such O-glycans have natural anti-Hp activities, which is a key factor in the protection of mucous cells from Hp attack (51). Then, how is the anti-Hp effect of O-glycan achieved? Through mass spectrometry and thin-layer chromatography (TLC), they found that the content of CGs in Hp is significantly lowered by adding O-glycans, meaning that the anti-Hp effect of O-glycan is achieved by interfering with the synthesis of CGs (51). This suggests that to resist Hp infection, we need to prevent CGT from damaging lipid rafts first. Nevertheless, up to now, there is still no anti-Hp drug with repairing lipid rafts as the therapeutic target in the world.

The addition of cholesterol and O-glycans can help clear Hp infection (50,51), and whether the mechanism is associated with the repair of the immune pathways on lipid rafts remains to be investigated. Recent studies have discovered that some Hp strains resistant to multiple antibiotics contain a very high level of CGs (52), and the study conducted by McGee *et al.* confirmed that such high levels of CGs can enhance Hp resistance to antibiotics (53).

Qaria *et al.* demonstrated that removal of CGs from the Hp surface can make some drug-resistant strains vulnerable to antibiotics (54). Although CGs synthesis is not the only antibiotic tolerance mechanism, CGs play a specific role in Hp resistance to various antibiotics.

Conclusions and outlook

Lipid rafts are key to persistent Hp infection, and a new field of research on pathogen-host cell interactions and signal transduction. The destruction of lipid rafts is of great Importance for understanding chronic gastritis, peptic ulcers, and stomach cancer from Hp persistent infection. Recent studies have revealed that lipid rafts are the transportation hubs of multiple inflammatory pathways (40-44), and Hp evades immune response by damaging lipid rafts to form persistent infections (48,49). The addition of cholestenone or O-glycan affecting lipid rafts has a significant effect on the inhibition of Hp persistent infection, which undoubtedly suggests that lipid rafts are effective drug targets against persistent Hp infection, thus important for eradicating persistent Hp infection and reducing the resistance of drug-resistant strains to antibiotics. The current studies suggest that cholestenone and O-glycan can control Hp infection by hindering the synthesis of CGs (50,51), and are therefore ideal targets for the treatment of persistent Hp infection. But if cholestenone and O-glycan can achieve this effect, why not use them as anti-Hp drugs? Are the antibacterial effects of these substances determined? What are the mechanisms that produce the effect other than those described above? Can they be used in combination with antibiotics to improve the efficacy of Hp eradication therapies? Are they good or bad for host cells? These questions all require further research and discussion.

At present, some achievements have been made in the drug research with lipid rafts as the therapeutic target (55). This means that it is also a very valuable research direction to target lipid rafts in the treatment of persistent infection of Hp. However, most of these experimental therapies are targeting destroying pathological lipid rafts, including examples of inflammarafts and clusters of apoptotic signaling molecule-enriched rafts (55). Drug research aimed at repairing damaged lipid rafts is relatively rare (55). This means that relevant researches are also significantly pioneering and innovative. Drugs repairing lipid rafts in persistent Hp infection may became a supplement to existing therapy.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-1000/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1000/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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