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Review

Cellular evasion strategies of *Helicobacter pylori* in regulating its intracellular fate

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ABSTRACT

Helicobacter pylori colonizes human stomach mucosa and its infection causes gastrointestinal diseases with variable severity. Bacterial infection stimulates autophagy, which is a part of innate immunity used to eliminate intracellular pathogens. Several intracellular bacteria have evolved multipronged strategies to circumvent this conserved system and thereby enhance their chance of intracellular survival. Nonetheless, studies on *H. pylori* have produced inconsistent results, showing either elevated or reduced clearance efficiency of intracellular bacteria through autophagy. In this review, we summarize recent studies on the mechanisms involved in autophagy induced by *H. pylori* and the fate of intracellular bacteria.

1. Introduction

The immune system of healthy individuals comprises potent mechanisms to protect the body against microbial infections. Autophagy is an evolutionarily conserved process to eliminate pathogens [1]. This catabolic process sequesters cytosolic components including misfolded proteins, injured organelles, and intracellular pathogens through a lysosome-dependent pathway [2]. Accumulated evidence shows that successful pathogens can hijack autophagy for their own replication [3]. This review focuses on a chronic gastric pathogen, *Helicobacter pylori,* which often inhabits mucosal layers and causes dyspeptic symptoms of varying severity. Findings of previous studies indicate that autophagy plays an important role in host defense, lessening *H. pylori* burden [4–7]. However, *H. pylori* is a facultative intracellular pathogen and is capable of invading and surviving not only in non-phagocytic cells but also in professional phagocytes [8–11]. In addition, *H. pylori* is detected in double-layered autophagosomes, indicating that autophagosomes serve as a peculiar niche for *H. pylori* multiplication to sustain intracellular infections [8,12,13]. However, the underlying mechanisms through which *H. pylori* coopts the autophagy machinery to affect bacterial clearance efficiency in host cells remain to be elucidated.

2. *H. pylori* virulence factors

2.1. Adhesion molecules

Microorganisms use bacterial adhesins to bind to host cells, protecting themselves from mechanical attack, such as acidic pH and mucosal liquids [14]. Genomic analysis reveals that *H pylori* contains more than 30 outer membrane protein (*omp*) genes including the *Helicobacter* OMPs (Hop) and the *hop*-related group (*hor*) [15]. The two most important adhesins in *H. pylori* are Lewis b (Leb) blood group antigen-binding adhesin (BabA) and sialyl Lewis X antigen-binding adhesin (SabA), and both these adhesins are Hop proteins [16–18]. Thus, the host and pathogen interact partly through the binding of adhesins to specific carbohydrate moieties of the gastric epithelium, promoting infection and inflammatory processes in the gastrointestinal tract.

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2.2. Vacuolating cytotoxin A (VacA)

VacA, which causes massive vacuolation in cultured cells, was first observed in culture supernatants of toxic *H. pylori* isolates [19]. Purification and subsequent studies on this unique toxin showed that VacA is synthesized as a 140-kDa polypeptide precursor, which undergoes trimming to yield a mature 95-kDa toxin and is secreted via an autotransporter encoded in its C-terminal domain [20–22]. It can assemble into water-soluble oligomeric forms including a single layer structure consisting of hexamers/heptamers and a double-layered assembly [23]. It is interesting that VacA disassembles at acidic pH, interacts with the lipid membrane and reassembles into a pore-forming hexameric structure in membranes, enabling the transport of chloride ions [22,24]. This unique channel-formation activity contributes to vacuolation in late endosomal and lysosomal compartments as well as increases in mitochondrial membrane permeability which leads to cytochrome *c* release [22,23].

Several surface molecules have been reported as VacA receptors. Receptor-like protein tyrosine phosphatase β (RPTPβ) binds to VacA through its terminal sialic acid domain in gastric epithelial cells [25,26]. RPTPα also serves as a functional VacA receptor in human kidney tumor cell G401 that lacks RPTPβ [27]. The interaction of VacA and RPTPα activates Src phosphorylation, resulting in CagA phosphorylation at Tyr972 in AZ-521 cells [28]. Notably, VacA is able to target the membrane of multiple cell types including gastric epithelial cells, T cells, and parietal cells. Therefore, VacA with high pore-forming activity is associated with increased disease severity [29]. It has been described that removing the membrane-associated region of VacA blocks its vacuolating activity and prevents VacA from integrating into the inner-mitochondrial membrane [30]. Recent evidence also shows that VacA binds to low-density lipoprotein receptor-related protein 1 (LRP1) and is involved in triggering autophagy as discussed below [31].

2.3. CagA and type IV secretion system (T4SS)

CagA is the gene product encoded within the 40-kb *cag*-pathogenicity island (*cag*-PAI) which also includes 31 additional genes that encode the T4SS [32]. The mechanisms that pathogens have exploited in order to introduce the virulence factors into the host cells. In another study conducted, *H. pylori cag*-T4SS interacts with the β1 integrin of the host cells in an arginine-glycine-aspartate (RGD)-independent manner, which initiates effector protein translocation [33]. When *H. pylori* contacts with the host membrane, CagA interacts with the externalized phosphatidylserine to facilitate its entry into target cells, which is important for the pathophysiological activity of CagA [34]. In addition to β1 integrin, a recent study by Königer *et. al.* describes that HopQ specifically interacts with human carcinoembryonic antigen-related cell adhesion molecule family (CEACAMs), facilitating CagA translocation into host cells [35]. These studies suggest that *H. pylori* exploits multiple ways to exert CagA translocation into the host cells. The translocated CagA can be phosphorylated and interacted with several host signaling proteins, thus interfering with host cell signaling [36]. It is known that CagA translocation and phosphorylation are mediated through cholesterol-rich microdomains present on the cytoplasm membrane (also referred as lipid rafts) [34,37–39]. Lipid rafts are important for efficient T4SS-mediated CagA translocation, inducing an elevated level of the cell hummingbird phenotype and increased IL-8 production [37]. The host membrane lipid phosphatidylserine plays a crucial role in CagA delivery and localization [34,40]. Moreover, simvastatin, a HMG-CoA reductase inhibitor, which reduces the level of cellular cholesterol and decreases translocation and phosphorylation of CagA, indicating that cholesterol is crucial for CagA-mediated actions [39].

2.4. Cholesterol-α-glucosyltransferase (CGT)

The *O*-Glycans of the human gastric mucosa diminish the levels of *H. pylori* infection by inhibiting the bacterial cholesterol-modifying enzyme, cholesterol-α-glucosyltransferase (CGT) encoded by the *type 1 capsular polysaccharide biosynthesis protein J* (*capJ*) [41]. CGT catalyzes the conversion of host cholesterol into cholesteryl-α-glucoside (αCG) [41]. Further modification of αCG yields cholesteryl-6′-*O*-acyl-α-glucoside, cholesteryl-6′-*O*-phosphatidyl-α-glucoside, and cholesteryl-6′-*O*-lysophosphatidyl-α-glucoside, which constitute a large proportion of the glycolipids in the cell wall [41]. Cholesterol glucosylation of *H. pylori* by CGT, interestingly, promotes *H. pylori* evasion of immune defenses [42]. We have previously shown that knockout of *capJ* (ΔCapJ), which abolishes the production of cholesteryl glucosides (CGs), greatly decreases the degree of T4SS-induced activities, including CagA translocation/phosphorylation, subsequent signaling events, the scattering hummingbird morphology in AGS cells, and IL-8 secretion [43]. This is mainly because CGs of the wild-type *H. pylori*-infected AGS cells are essential for reorganizing the lipid-raft membranes for efficient T4SS function [43]. Furthermore, cholesterol-glucosylated *H. pylori* is largely internalized via a lipid-raft-dependent pathway, which delays the maturation of phagosomes and facilitates the evasion of macrophages [44], suggesting a role in interfering with autophagy, which is discussed in a later section [45]. *H. pylori* harboring CGT, inhibits interferon gamma-induced signaling, which avoids host inflammatory responses [46]. Noticeably, CGs also contribute to cell wall integrity, morphology, and antibiotic resistance [47]. Collectively, these findings highlight the multiple strategies employed by *H. pylori* for persistent colonization.

2.5. Other virulence factors

Other virulence factors are also engaged in *H. pylori*-induced pathogenesis, such as urease, flagella, and heat-shock proteins (Hsp). Infection of *H. pylori* upholds the periplasmic pH to adapt to the acidic environment of stomach by means of urease [48]. The increased levels of ammonia from hydrolysis of urea by urease as well as the metabolites from recruited neutrophils disrupts the gastric epithelium, which synergistically facilitates the development of gastric malignancies [49,50]. In addition, *H. pylori* possesses four to eight unipolar flagella that are important in bacterial colonization and induce inflammatory response [51]. The flagellar sheath of *H. pylori* provides the protection of acid-labile flagellar structure from the attack of the acid environment [52]. Additionally, *H. pylori* harbors two main heat-shock proteins, GroES-like HspA (Hsp10), and GroEL-like HspB (Hsp60). The regulation of heat-shock proteins expression by *H. pylori* is crucial for bacteria to adapt and survive in the hostile environment of the stomach [53].

3. Autophagy in bacterial infection and pathogenesis

3.1. Composition, structure, and functions of autophagy

Autophagy is an intracellular degradation process by which cytoplasmic constituents are degraded by the lysosome [54]. The three major forms of autophagy include microautophagy, macroautophagy, and chaperone-mediated autophagy [55,56]. Microautophagy and chaperone-mediated autophagy are directly recruited to a lysosome, which engulfs small portions of cytosol and receives chaperone-associated cargo [57]. Macroautophagy can sequester cytosolic molecules, including damaged organelles or pathogens, in the autophagosome [57]. In mammalian cells, macroautophagy occurs in the cytoplasm; the organelles are surrounded by a phagophore or cytoplasmic double-membrane-bound structure, which start growing at both ends to create an autophagosome [58,59]. The autophagosome subsequently fuses with a lysosome, forming a single, large, and membrane-surrounded vesicle (autophagolysosome); both the membrane and its contents are

Table 1
Bacterial utilization of host autophagy for their intracellular survival and pathogenesis.

Bacteria growth restricted by autophagy components	Bacteria evades being targeted by host's autophagy	Bacteria exploit autophagy components for its replication and pathogenesis
Mycobacterium tuberculosis [97]	*Shigella flexneri* [119]	*Brucella abortus* [120]
Mycobacterium marinum [121]	*Listeria monocytogenes* [122]	*Coxiella burnetiid* [123]
Group A Streptococcus [124]	*Burkholderia pseudomallei* [125]	*Legionella pneumophila* [126]
Salmonella enterica serovar Typhimurium [127]		*Staphylococcus aureus* [128]
		Francisella tularensis [129]

then degraded by lytic enzymes [60,61]. Autophagy has been demonstrated to play crucial roles in biological processes, such as cell protection, response to starvation, recycling nutrients from digested organelles and macromolecules by removing damaged organelles and aberrantly folded proteins, diseases, and host defenses [62].

3.2. Bacterial infection and the autophagy pathway

The large-scale degradation of invading pathogens by autophagy, called xenophagy, has been commonly used to describe the host defense response to effectively eliminate the invading pathogens including intracellular bacteria [63]. Various studies have been conducted on different pathogens and how their virulence factors manipulate the autophagy pathways of their hosts to promote their intracellular survival and multiplication. Examples of such pathogens include *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, *Shigella flexneri*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Legionella pneumophila*, all of which have been extensively studied to elucidate the strategies they use to alter the host autophagy pathway (Table 1). The details of the underlying mechanism will be covered later.

3.3. Adhesion and invasion of cells

Pathogenic microbes exhibit numerous approaches for evading immune defenses and developing a protected niche within the host. Therefore, the interplay between the initial entry of these pathogenic microbes into the host cells and the response of the host cells plays a critical role in determining the fate of the microbes. Wang and Hajishengallis previously reported that *Porphyromonas gingivalis* relies on the lipid rafts of the macrophages to enhance its intracellular bacterial load and survival [64]. There is mounting evidence that shows parasites [65–67], viruses [68,69], and prions [70,71] target the lipid rafts of the host cells, which benefits infections. Lipids cluster together to form microdomains in cell membranes, i.e., lipid rafts. Lipid rafts composed of high concentrations of cholesterol and glycosphingolipids [72], that modulate a wide array of cellular processes such as signal transduction [73], membrane trafficking [74,75], and cytoskeletal organization [76,77].

Various studies have been shown that lipid rafts are common platforms for bacteria to adhere and invade to host cells [78]. A study conducted by Shin et al. demonstrated that lipid rafts serve as important docking points for bacteria that express FimH [79]. Furthermore, these bacteria reside in vacuoles containing the lipid rafts, which is important for promoting their intracellular survival. *H. pylori* also exploits lipid rafts for initial adhesion and invasion. A study conducted by Wunder et al. demonstrated that *H. pylori* is auxotrophic for cholesterol and is able to extract cholesterol from the lipid rafts of the host, resulting in immune evasion [42]. This study also corroborates our previous findings that lipid rafts of the epithelial cells play a key role in *H. pylori* internalization [37]. In addition to bacteria, viruses also rely on lipid rafts for attachment and invasion. The coronavirus, infectious bronchitis virus is reliant on the host's lipid rafts for attachment and thereby promoting viral infection [80].

3.4. Cytoplasmic trafficking

Upon invasion of host cells by the pathogens, they can modulate the host cell trafficking machinery to promote evasion from the host innate immune response through Rab GTPases which are proteins responsible for vesicular trafficking, at least in part [81]. Rab proteins, when activated, interact with a large number of Rab effector proteins to regulate different cellular functions such as membrane trafficking and intracellular signaling [82]. Rab proteins reside in intracellular membrane compartments of the plasma membrane, mitochondria, and the nucleus and are involved in a large number of cellular functions [83]. Above all, Rab proteins are known for their roles in the endocytic/exocytic pathways, the retrograde/anterograde secretory pathways, and membrane recycling [84].

As xenophagy involves the trafficking of pathogens in the host cells to the lysosomes for degradation, various studies have demonstrated that different pathogens target different Rab proteins to promote their intracellular survival. For instance, *M. tuberculosis* modifies Rab22, which is associated with the maturation of phagosome [85], and promotes the accumulation of Rab14 in *M. tuberculosis* containing phagosomes, preventing fusion of the phagosome with lysosome [86].

While Rab proteins serve as one of the key targets for pathogens, other molecules are also triggered by pathogens to manipulate the host's cytoplasmic trafficking. *Salmonella* secretes secretor effector protein (SifA) via its type 3 secretion system (T3SS) from the *Salmonella*-containing vacuoles to the cytoplasm [87]. SifA then binds to kinesin-interacting protein (SKIP), a host protein, which downregulates the recruitment of kinesin to the bacterial vacuoles, thereby promoting the membrane dynamics of vacuoles [88]. Upon invading the host cells, a few pathogens escape from the vacuoles and replicate in the host cytoplasm. These pathogens express factors that initiate the process of actin polymerization, facilitating their propagation in the cells. For example, *L. monocytogenes* secretes the virulence factor listeriolysin O (Llo), a pore-forming exotoxin which degrades the membrane of internalized vacuoles in the infected host cells [89], eventually resulting in vacuolar dissolution, and thereby promoting intracellular survival [90]. Furthermore, the pathogen is able to translocate across the infected cell wall to spread and invade adjacent cells through actin-based motility [91]. This phenomenon is attributable to the presence of the protein actin assembly-inducing protein (ActA) on the bacterial surface. ActA acts as a nucleator, which induces actin polymerization allowing the bacteria to move forward in the host cells [92].

3.5. Bacterial survival or elimination in host cells

Upon entering host cells, several pathogens evade phagosome fusion with the degradative lysosome through different mechanisms thereby increasing their survival and bacterial load in the host cells. One of the mechanisms by which pathogens promote their intracellular survival is by arresting phagosome maturation. To achieve this, pathogens are capable of obstructing phagosomal lipid metabolism. One example is *M. tuberculosis*, which produces a phosphatase identified as secreted acid phosphatase (SapM). SapM is capable of converting PI(3)P to PI, inhibiting late phagosome endosome fusion which in turn inhibits phagosomal maturation [93]. Another example is *Salmonella enterica*,

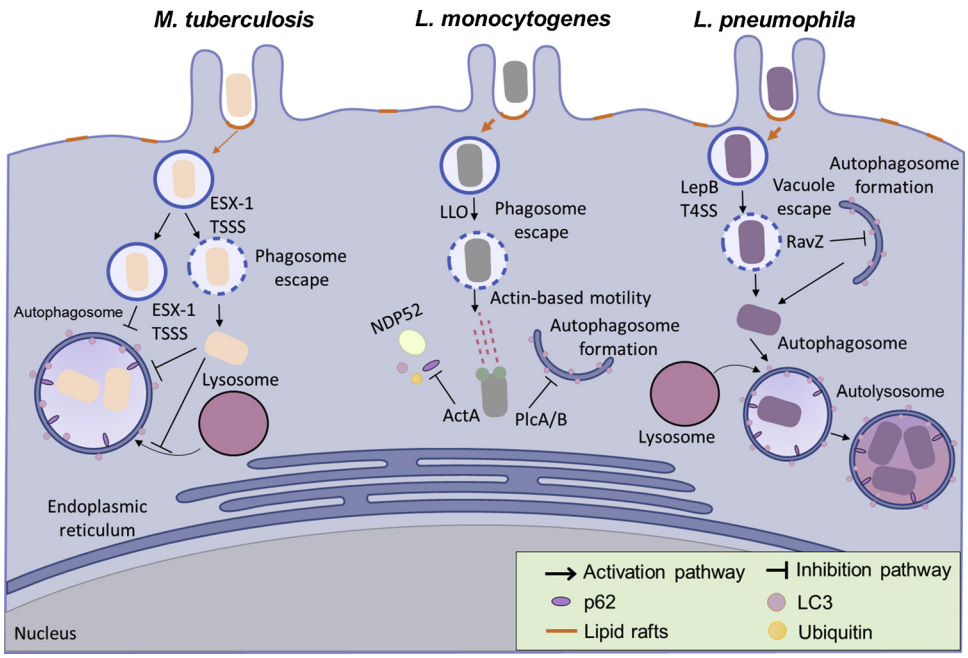

Fig. 1. Bacteria hijack lipid rafts to induce autophagy as part of their infectious strategy. *Mycobacterium tuberculosis* is capable of surviving in the phagosome after being internalized into the host cell by preventing phagosomal maturation and inhibiting autophagosome fusion with lysosome. *M. tuberculosis* is able to survive and replicate in the phagosome. However, cytosolic translocation of *M. tuberculosis* can occur when it expresses the early secretory antigenic target of *M. tuberculosis* (ESAT-6) secretion system (ESX-1) and type VII secretion system (TSSS). *Listeria monocytogenes* employs various virulence factors in its escape from autophagy. Upon escaping from the phagosome using its pore forming toxin (Listeriolysin O), *L. monocytogenes* expresses another virulence factor ActA to inhibit the recruitment of p62 and NDP52 to its surface. Another group of virulence factors, two phospholipases C (PlcA/B) then prevent the formation of the autophagosomal membrane. In *Legionella* infections, *L. pneumophila* escapes from the vacuole and enters the host's cytosol via LepB using a type IV secretion system. The cytosolic *L. pneumophila* is subsequently recognized by the autophagic machinery but delays phagocytosis by using its effector protein RavZ. In the autophagosome *L. pneumophila* also delays the fusion of the autophagosome with lysosome to allow it time to develop into an acid-resistant form. This acid-resistant form of *L. pneumophila* can then replicate in the acidic autophagolysosome.

which injects effector sigma D factor (SigD) into host cells, leading to the modulation of phosphatidylinositol 3-kinase (VPs34) and increasing vacuolar PI(3)P, thereby averting phagosome maturation and preventing fusion with a lysosome [94].

A second method used by pathogens to evade host cell degradation is escaping from the host cell phagosome. Studies on *Mycobacteria* species have shown that they activate host cytosolic phospholipase A2 to assist the microbe in escaping the phagosome [95]. This clearly demonstrates the highly flexible adaptation strategy of *Mycobacteria* for surviving in host cells (Fig. 1). Recently, another survival strategy employed by the pathogen has been discovered. While employing this strategy, the pathogen directly interferes with the maturation of the autolysosome. In a very recent study conducted by Zhang et al., *H. pylori* was able to inhibit lysosomal function and suppress the process of autolysosomal maturation, thereby circumventing autophagic degradation [96].

In contrast to the abovementioned examples where pathogens subvert autophagy to promote their intracellular survival, some pathogens that are eliminated by activating autophagy. Gutierrez et al. demonstrated that activation of autophagy by starvation or treatment with rapamycin triggers an effective clearance of *M. tuberculosis* in macrophages [97]. Similarly, using rapamycin to activate autophagy led to an enhancement of targeting *Burkholderia cepacia* to lysosomal compartments, resulting in better prognosis in patients with cystic fibrosis [98]. Notably, a new LC3-associated phagocytosis (LAP) autophagic machinery has been recently identified [99]. The key function of LAP is to enhance fusion of phagosomes and lysosomes, which promotes pathogen killing [100]. However, the detailed mechanism by which LAP enhances phago-lysosome fusion remains to be studied.

4. *H. pylori* modulates autophagy

Autophagy as an intracellular defense to eliminate pathogens can be induced by *H. pylori* in both gastric epithelial cells [13,96,101,102] and professional phagocytes [103,104]. Recently accumulating evidence has shown that *H. pylori* exploits host autophagy to survive within cells and even multiply [10,105,106]. However, the mechanisms vary depending on the host cells or bacterial strains. In this section, we summarize recent studies conducted to elucidate the different mechanisms of autophagy of *H. pylori* in gastric epithelial cells and professional phagocytes.

4.1. *H. pylori infects gastric epithelial cells*

Several virulence factors and host genes have been shown to affect *H. pylori*-induced autophagy (Table 2). Of these, VacA was first reported by Terebiznik et al. to induce autophagy in the AGS cell line [101]. *H. pylori* infection of AGS cells increases LC3 puncta and promotes the conversion of LC3-I to LC3-II. Moreover, formation of LC3 puncta was decreased in *atg5*$^{-/-}$ mouse embryonic fibroblasts (MEFs) challenged with *H. pylori*, indicating that *H. pylori*-induced autophagy in AGS cells follows the canonical pathway [101]. Treatment of AGS cells with purified toxin or conditioned culture media supernatants revealed that VacA but not CagA, CagE, urease, or the T4SS is essential and sufficient to induce autophagy [101]. In contrast, another study using AZ-521 cells found that treatment with 3-methyladenine (3-MA), a PtdIns3K inhibitor, or knockdown of BECN1 did not inhibit VacA-induced autophagy, suggesting that it does not use the canonical pathway [31]. This finding contradicts the results in AGS cells reported by Tang et al. [102], possibly due to the different cell lines used. Interestingly, autophagy in turns modulates the activity of intracellular VacA, indicating that autophagy protects host cells against *H. pylori* infection. However, the mechanism by which these virulence factors induce autophagy remains to be investigated. One clue is that VacA damages mitochondrial function and causes nutrient depletion which may be linked to inhibition of mTOR signaling and induction of autophagy [107].

Many studies have reported that *H. pylori* can prevent its degradation, allowing it to reside and multiply in autophagosomes. Raju *et. al.* showed that prolonged exposure (24 h) to a toxin disrupts subsequent autophagosome-lysosome fusion and causes the accumulation of autophagsomes in the cells [108]. This observation suggests that the underlying mechanisms of autophagy are not the same in acute and chronic exposure (Fig. 2). The final step of autophagy is formation of autolysosomes, which are the fusion of autophagosomes and lysosomes. For instance, after prolonged exposure to conditioned culture media, AGS cells show a reduced level of the lysosomal protease cathepsin D,

Table 2
H. pylori manipulates autophagy in phagocytic and non-phagocytic cells.

Bacterial virulence factors (V) or host determinants (H)	Factor	Cell line used	Role in intracellular survival and/or autophagic processes	Reference
Epithelial cells				
V	CagA	AGS	Does not involve in *H. pylori*-induced autophagy	[101]
			Promote *H. pylori* intracellular survival	[13]
	CagE	AGS	Does not involve in *H. pylori*-induced autophagy	[101]
	VacA	AGS	VacA channel-forming activity is essentitial for the intudtion of autophagy	[101]
		AZ521	Induce autophagy by binding to LRP1	[31]
		AGS	Prolong expossure may cause autophagy disruption due to lack of cathepsin D in lysosome	[107]
		AGS	Inhibit *H. pylori* internalization and invasion	[13]
	BabA	AGS	Promote *H. pylori* intracellular survival	[13]
	Urease	AGS	Does not involve in *H. pylori*-induced autophagy	[101]
H	ATG5	AGS	Essential for *H. pylori*-induced autophagy	[101]
		AGS	Decrease the intracellular survival of VacA+ *H. pylori*	[107]
	ATG12	AGS	Essential for *H. pylori*-induced autophagy	[101,102,107]
	ATG16L1	AGS	Single nucleotide polymorphism ATG16L1300A may increase the intracellular survival of *H. pylori*	[107]
	BECN1	AGS	Essential for *H. pylori*-induced autophagy	[102]
		AZ521	Does not involve in VacA-induced autophagy	[31]
		HFE145	Promote H. pylori intracellular survival by inhibiting autophagosome formation	[96]
	LRP1	AZ521	Induce autophagy by binding to VacA	[31]
	MIR30B	AGS	Inhibit *H. pylori*-induced autophagy by downregulating ATG12 and BECN1	[102]
	MIR30D	AGS, GES1	Inhibit *H. pylori*-induced autophagy by downregulating genes involved in autophagy pathway	[112]
	PtdIns3K	AZ521	Does not involve in VacA-induced autophagy	[31]
Professional phagocytes				
V	CagA	THP-1	Promote *H. pylori* internalization and intracellular survival	[103]
		BMDCs	Promote *H. pylori* intracellular survival	[104]
	VacA	THP-1	Promote *H. pylori* internalization and intracellular survival	[103]
		BMDCs	Promote *H. pylori* intracellular survival	[104]
	CapJ	J774A.1	Delay the fusion of autophagosome and lysosome	[45]
		J774A.1	Delay *H. pylori* internalization	[44]
H	ATG16L1	PMBCs	Single nucleotide polymorphism ATG16L1300A may increase the intracellular survival of *H. pylori*	[107]
	PtdIns3K	THP-1	Inhibit the bacterial multiplication	[103]
		RAW264.7	Does not inhibit the bacterial multiplication	[103]

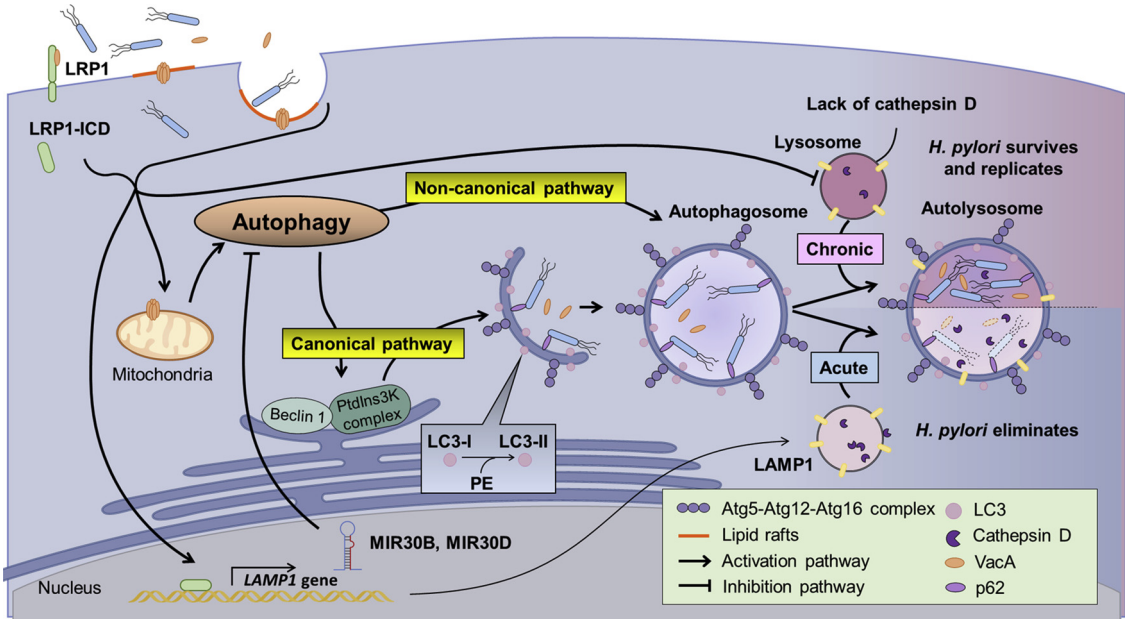

Fig. 2. *H. pylori* modulates autophagy in epithelial cells. VacA is an essential virulence factor for modulating *H. pylori* induced autophagy via various mechanisms. The initiation of infection starts with the internalization of *H. pylori* and VacA, the formation of VacA pores or the binding of VacA to LRP1. The internalization of *H. pylori* and VacA may induce autophagy. VacA forms pores in the mitochondria, causing nutrient depletion which induces autophagy, mediated through the inhibition of mammalian target of rapamycin complex 1 (mTORC1). Autophagy may proceed through either the canonical or non-canonical pathway depending on the cell lines used. In addition to inducing autophagy, *H. pylori* can hijack the autophagy process. *H. pylori* infection may induce the expression of MIR30B and MIR30D, which subsequently inhibit autophagy by targeting different autophagy-related genes. Also, the acidification of lysosome is disrupted by chronic exposure to VacA, causing the accumulation of pro-cathepsin D. These mechanisms allow *H. pylori* to survive and replicate in the autophagosome.

due to disruption of lysosome acidification [108]. The defective autophagosomes in AGS cells caused by VacA may lead to the accumulation of SQSTM1, a major cargo ubiquitin-binding receptor. This phenomenon is also observed in patient biopsies [108]. Other reports also showed the accumulation of different proteins due to VacA-disrupted autophagy, leading to changes in cellular ROS levels [109] and gene mutations [110]. The accumulation of p62 activates nuclear factor erythroid 2-related factor (NRF2) which controls the expression of ROS detoxification genes in AGS cells [108,110]. A contradictory result showed that CagA and HspB, but not VacA, regulate the antioxidant response [111]. Therefore, a detailed investigation of the effect of p62 and NRF2 on *H. pylori* virulence factors that regulate autophagy is warranted.

Consistent with the previous findings, a recent study has also shown reduced activity of lysosomal proteases including cathepsin D, N-acetyl-β-D-glucosaminidase (β-NAG), and acid phosphatase in HFE-145 cells infected by *H. pylori* [96]. Disruption of lysosome acidification causes the repression of the retrograde trafficking of mannose-6-phosphate receptors which play an important role in carrying lysosomal hydrolytic enzymes in the trans-Golgi network [96]. However, the mechanism underlying the disruption of lysosome acidification in *H. pylori* infected cells is still unclear.

The internalization of VacA or *H. pylori* plays a critical role at the beginning of autophagy. There is evidence that VacA induces autophagy by binding to LRP1 [31]. Interestingly, s1m1 VacA, but not s1m2 or s2m2 interacts with LRP1 and stimulates autophagy [31]. Moreover, knockdown of LRP1 significantly reduces autophagy, indicating that VacA internalization is crucial for the induction of autophagy. Internalized VacA may further alter the membrane permeability of mitochondria, causing the release of cytochrome *c* and leading to the induction of mitophagy and apoptosis [29,111]. In addition, the internalization of *H. pylori* is unaffected by VacA and CagA, but is correlated with *H. pylori* invasin NudA in AGS cells [112]. Notably, the internalization of *H. pylori* also requires the activation of PI3-kinase and PKC in AGS cells [105].

In addition, although VacA induces autophagy, *H. pylori* may evade autophagy process by altering the expression of various microRNAs, including MIR30B and MIR30D [102,112]. *H. pylori* infection may increase the expression of MIR30B in different gastric cell lines (i.e. MIR30D in AGS and GES1 cells). Both MIR30B and MIR30D target several genes involved in autophagy, such as ATG12 and BECN1. Interestingly, a very recent report by Tsugawa et al. showed that overexpression of capping actin protein of muscle Z-line alpha subunit 1 (CAPZA1) blocks the CagA-degraded autophagy by downregulating the expression of lysosomal associated membrane protein 1 (LAMP1) in *H. pylori*-infected cells [113]. Moreover, Tsugawa et al. showed that CagA accumulation in CAPZA1-overexpressing cells is essential for the upregulation of CD44v9 which is a cancer stem-cell marker, suggesting that overexpression of CAPZA1 could be a key risk factor in gastric cancer development [114]. This notion is supported by an earlier study that the abundance of CD44v9 is a superior prognostic predictor than the presence of open-type gastric mucosal atrophy for the recurrence of gastric cancer in patients after the resection of early gastric cancer [115]. These lines of evidence together suggest that *H. pylori*-infected gastric mucosa exhibits negative regulation of autophagy through an orchestrated array of actions from multiple virulence factors. As such, *H. pylori*-mediated defective autophagy links to an increased gastric cancer risk, thereby promoting the development of various gastrointestinal diseases.

4.2. H. pylori manipulates autophagy in professional phagocytes

Compared to gastric epithelial cells, the effects of *H. pylori*-induced autophagy by professional phagocytes are much more complicated due to differences between the cell lines and bacterial strains used. The primary role of phagocytes is to ensure the efficient clearance of harmful foreign particles, bacteria, and dead or dying cells. In the case of *H. pylori* infection, various studies have demonstrated its capability to delay phagocytosis by phagocytes.

Wang *et. al.* first reported that *H. pylori* induces activation of autophagy in macrophages [103]. In another previous study, *H. pylori* infection was found to increase the number of LC3-puncta in both RAW264.7 and THP-1 cells [12]. In addition to activation of autophagy, Wang *et. al.* further demonstrated that *H. pylori* multiplies in human monocytic THP-1 or U937 cells, but not in murine macrophage RAW264.7 cells. Another report showed that *H. pylori* could replicate in the autophagosomes of mouse bone marrow-derived dendritic cells (BMDCs) [104]. Moreover, *H. pylori* infection of BMDCs may impair MHC class II molecule surface expression which is negatively regulated by Toll-like receptor (TLR)-2 and 4. The correlation of TLR4 polymorphism and gastric cancer may deserve further attention [116]. Interestingly, both studies show that CagA and VacA are important for the survival and multiplication of *H. pylori* in autophagosomes [103,104]. These results contradicted the observations in gastric epithelial cells, which may be due to the different types of cell lines and *H. pylori* strains used.

This observation corroborates with another study where pre-loading *H. pylori* with cholesterol enhances the rate of *H. pylori* internalization [117]. We recently have shown that CGT, a main factor in *H. pylori* cholesterol glucosylation, promotes autophagosome formation, and simultaneously inhibits lysosome formation in murine macrophages to improve its intracellular survival [45]. This study also demonstrated that ATG12 is involved in the autophagy pathway, which is essential for *H. pylori* intracellular survival [45]. In addition, treatment of macrophages with simvastatin promoted autophagy and thereby reduced intracellular bacterial burden [7] (Fig. 3).

H. pylori induced autophagy in phagocytes is affected by the host genotype. For instance, several gene polymorphisms are associated with Crohn disease, an inflammatory bowel disorder, which affects *H. pylori* induced autophagy. ATG16L1, which is involved in autophagosome formation with the T300A mutation makes host more susceptible to *H. pylori* infection and reduces *H. pylori*-induced autophagy [108]. However, the detailed underlying mechanism remains to be explored. Another gene involved in this autophagy is the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor. During *H. pylori* infection, NOD2 activates nuclear factor-kappa B (NF-κB) signaling which induces inflammation. However, the R702W mutant cannot activate NF-κB signaling [118]. Therefore, the mutation is associated with gastric MALT lymphoma, which is usually associated with *H. pylori* infection [118]. In addition to NOD2, TLR4, a pattern recognition receptor, also plays an important role in stimulating macrophage autophagy by binding to lipopolysaccharides on the bacteria [7,45]. TLR4 polymorphisms have been shown to correlate with gastric cancer [116]. However, further experiments are needed to reveal the underlying mechanisms.

5. Conclusions and perspectives

Autophagy is increasingly recognized as a vital route for host defense. Thus, it is not surprising that successful intracellular pathogens are evolved to dysregulate autophagy, contributing to disease pathogenesis. *H. pylori*, a small pathogen, shows remarkable capabilities to enhance its intracellular survival and propagation by means of a limited number of virulence factors, exemplified by multiple strategies that have evolved to negatively regulate autophagy. This, in turn, increases the risk of developing *H. pylori*-associated diseases. However, there are discrepancies in the results obtained to date, mainly due to the different types of cell models and/or bacterial strains used. Furthermore, the connection between virulence factors, dysregulated intracellular trafficking, disease etiology and its clinical relevance remains largely unanswered. For instance, does CagA, VacA, or CGT mediate membrane remodeling and interfere with intracellular trafficking, and how does

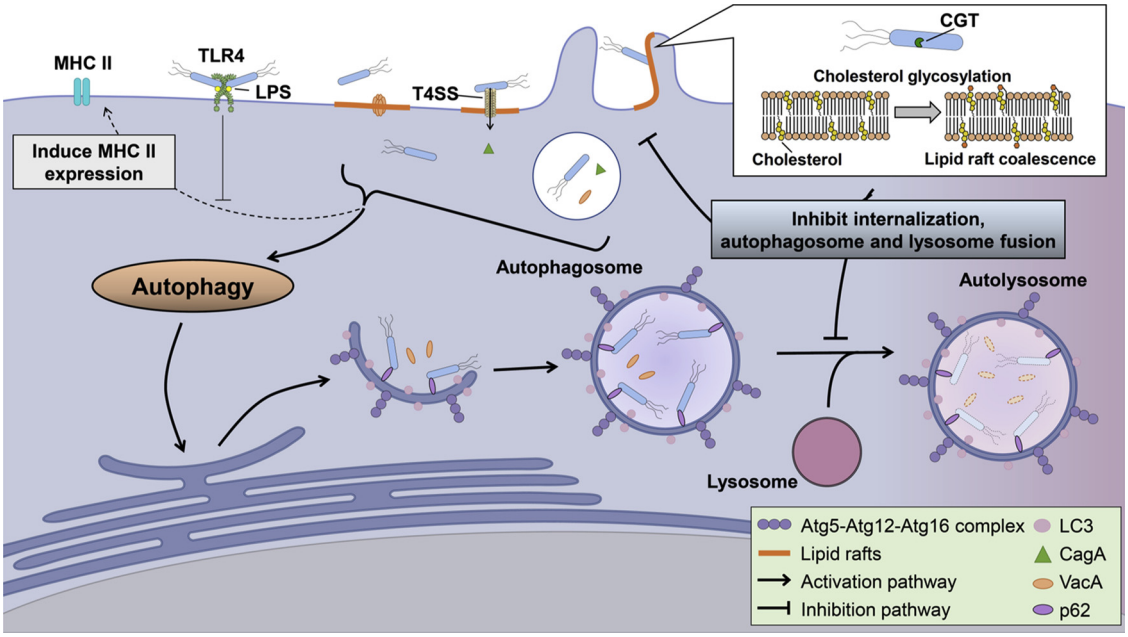

Fig. 3. *H. pylori* regulates autophagy in professional phagocytes. Unlike epithelial cells, both VacA, CagA and T4SS play an important role in *H. pylori*-induced autophagy. Recent studies have shown that HP0421, a cholesterol-α-glucosyltransferase (CGT), modulates autophagy and promotes bacterial survival in macrophages by clustering lipid rafts. The disrupted lipid rafts result in a lower internalization of *H. pylori* and interfere with autophagosome fusion with lysosomes. Another study has shown that *H. pylori* infection may increase the expression of major histocompatibility complex II (MHC II). However, TLR4 plays a negative role on BMDC maturation by inhibiting this process.

this relate to a defective autophagy? Although VacA-induced autophagy is dependent on LRP1, the detailed mechanisms and signaling underlying the autophagic trafficking remains to be clarified. Negative regulation of autophagy in *H. pylori*-infected gastric biopsies harboring abundance of CAPZA1, accumulation of CagA, and expression of CD44v9 is considered to increase gastric cancer risk. It will be of interest to characterize CagA accumulation in CD44v9-negative gastric epithelial cells. It is important to examine these data using cell models, *in vivo* studies, and in clinical settings. Further investigations are required to provide a mechanistic basis for better understanding the host-pathogen interplay in the context of autophagy and disease progression. This may facilitate the development of new strategies for combating diseases affected by infection and autophagy.

Declaration of Competing Interest

The authors did not have conflicts of interest to declare for this work.

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