

Review

Helicobacter pylori Vacuolating Cytotoxin, VacA

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SUMMARY: *Helicobacter pylori* is the leading bacterial cause of food-borne illness worldwide and plays a major role in the development of chronic gastritis, peptic ulcer, and gastric cancer. Strains isolated from patients contain the *cagA* gene (cytotoxin-associated gene A) and produce the vacuolating cytotoxin, VacA. Recent molecular and cellular studies of VacA action have begun to unravel its structure and the details of the mechanism of gastric injury caused by *H. pylori* infection.

1. Introduction

Helicobacter pylori is a motile, spiral-shaped, and microaerophilic Gram-negative bacterium, adapted to colonize and survive in the stomach mucosa; it is believed to colonize more than 50% of the world population (1-3). Although *H. pylori* generally survives within the gastric mucus layer, it can attach to host cells. *H. pylori* can use at least five different adhesins for successful attachment to gastric epithelial cells. Adaptation requires expression of several adhesins and of

a potent urease to neutralize gastric acidity by producing ammonia from urea present in mucosal secretions (4,5).

H. pylori is believed to be a major cause of chronic active gastritis, peptic ulcer, and atrophic gastritis; it is associated with an increased risk of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. As shown in Fig. 1, *H. pylori* infection is responsible for human diseases, and is acquired mostly in childhood and persists chronically when untreated. Persistent *H. pylori* infection results in the induction of an inflammatory response and epithelial cell

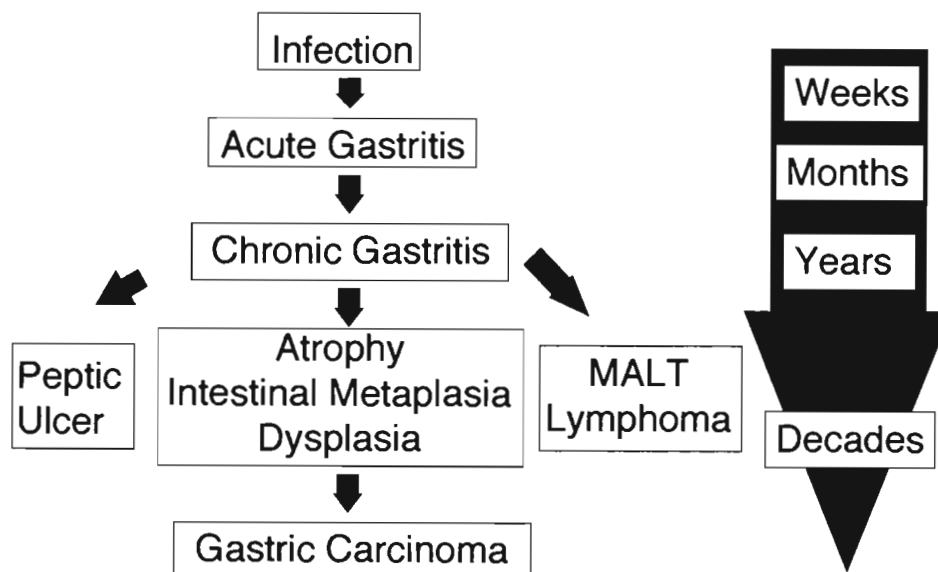


Fig. 1. *H. pylori* is responsible for human disease. *H. pylori* infection induces diseases listed in the boxes years or decades later as shown by time scale on the right-hand side (6).

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damage, which is manifested histologically as chronic superficial gastritis. Although most infections remain asymptomatic, about 10% of *H. pylori*-infected people become ill, suffering from peptic ulcer, gastric adenocarcinoma, and/or MALT lymphoma, years or decades later (6).

Intracellular vacuolization has been observed by electron microscopy of gastric epithelial cells from patients with chronic gastritis infected with *H. pylori* (7,8). *H. pylori* produces and secretes a protein toxin, the vacuolating cytotoxin, VacA, which induces cytoplasmic vacuolation in some eukaryotic cells, leading eventually to cell destruction. So far, VacA is the only protein toxin known to be produced by *H. pylori*. Before purification and characterization of VacA, an immunodominant protein encoded by the *cagA* gene (cytotoxin-associated gene A), which is located at the border of a pathogenicity island of *H. pylori*, was mistaken for VacA.

Although the function of CagA, which is translocated into the host cell leading to tyrosine-phosphorylation (9-11), is still unknown, type I strains, which produce both VacA and CagA, are considered more pathogenic and are associated with the more severe diseases (i.e., ulceration, adenocarcinoma) than the type II strains lacking VacA activity and the pathogenicity island (12).

Although a clear functional association between VacA and the clinical outcome of any type of gastroduodenal disease cannot be drawn yet, VacA has been proven epidemiologically to be a virulence factor associated with peptic ulcer (13,14). In addition, oral administration of VacA causes gastric mucosal damage in mice, suggesting that the action of this toxin may contribute to epithelial cell injury or peptic ulceration in *H.*

pylori-infected humans (15-17).

2. Structure and biological activity

In 1988, Leunk et al. first reported the presence of a cytotoxin with vacuolating activity in culture filtrates of *H. pylori* (18). In 1992, Cover and Blaser reported the purification of VacA from a culture supernatant (19) and its sequencing revealed a 139-kDa protein (20). As shown in Fig. 2, the 87-kDa mature toxin is generated by proteolytic cleavage of a 140-kDa precursor at the bacterial outer membrane (21).

Purified VacA behaved as a molecule of about 90 kDa under denaturing conditions, whereas the native toxin was an oligomeric complex of about 1,000 kDa, suggesting self-association. Two different VacA genotypes, m1 and m2, have been found in clinical isolates of *H. pylori* (22,23). The m2 genotype is distinguished from the m1 genotype VacA by the presence of a unique middle-region locus. The m1 and m2 proteins differ in cell specificity, suggesting that the two VacA molecules have different receptor-binding characteristics (24).

VacA of the m1 genotype is an oligomeric toxin, composed 6 or 7 subunits. The monomer of VacA is sometimes proteolytically cleaved into two fragments of about 37 and 58 kDa after secretion. The amino-terminal 37-kDa fragment is the enzymatically active "A portion" and the carboxy-terminal 58-kDa fragment is the receptor-binding "B portion" like other bacterial "AB toxin"s (25-27). Within several hours of the addition of VacA to human gastric adenocarcinoma, AZ-521 cells, large intracellular vacuoles with an acidic intravacuolar pH, become visible, as shown in Fig. 3. In

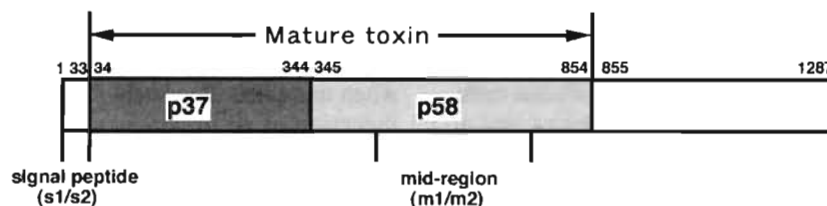


Fig. 2. Structural organization of VacA produced by *H. pylori* (ATCC49503 strain). VacA of strain ATCC49503 consists of 1287-amino acids, and undergoes cleavage of a 33-amino acid amino-terminal signal sequence and a 400-amino acid carboxy-terminal peptides sequence to yield a mature secreted toxin (821 amino acids). Two major types of signal sequences and mid-regions were detected, designated as s1 and s2, and m1 and m2, respectively. The mid-region spans approximately 300 amino acids, and m1 and m2 sequences have about 55% amino acid identity in this region.

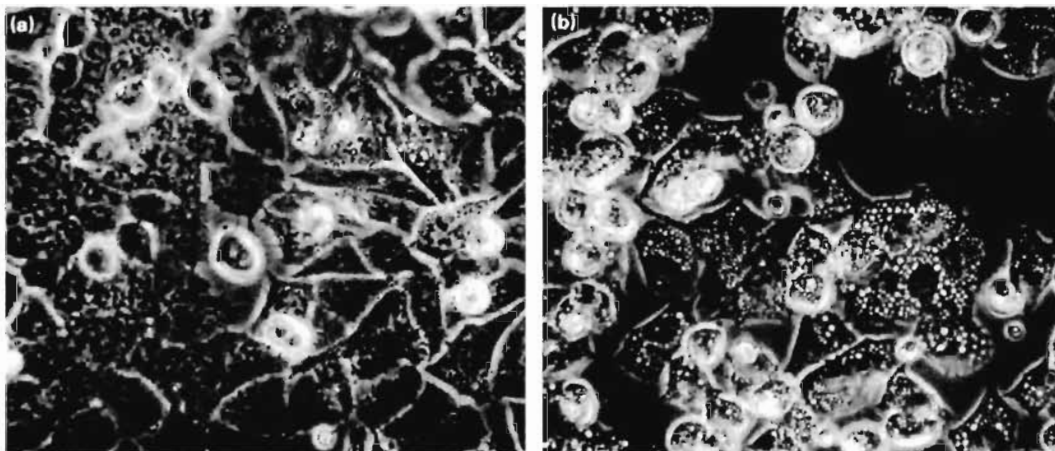


Fig. 3. Morphological change of human gastric adenocarcinoma cell line AZ-521 cells caused by VacA using phase-contrast microscope. All photographs were taken at a magnification of x 400. (a) Control, (b) AZ-521 cells treated with 120 nM VacA for 24 h.

general, to quantify the vacuolating activity of VacA, the uptake of neutral red into the vacuoles in VacA-treated cells was determined by subtracting the absorbance of cells incubated without toxin from that of toxin-treated cells.

Incubation of VacA at pH 2 increased its vacuolating activity, and its resistance to acid and pepsin degradation. It was notable that acid-activated VacA contributed to the development of duodenal lesions, independent of *H. pylori* (28). In addition, VacA was activated not only under acidic but also alkaline conditions resulting in its enhanced binding to target cells (29). Although the detailed mechanism is the subject of much speculation, taken together with the recent report on the tertiary structure of VacA, the acid- and alkali-activation of VacA might reflect a molecular change from hexamer to heptamer, resulting in the exposure of some portion of the protein surface needed for receptor binding (29).

3. Receptor

VacA binding to specific high-affinity cell surface receptors was shown by using indirect immunofluorescence and flow cytometry; high-affinity toxin binding was necessary for cell intoxication (30,31). A 250-kDa receptor protein tyrosine phosphatase (RPTP) β served as a receptor for VacA on AZ-521 cells; a second protein, p140, was also commonly detected in VacA-susceptible cells (29,32,33). The overexpression of RPTP β conferred VacA sensitivity on BHK-21 cells transfected with the RPTP β cDNA, consistent with RPTP β acting as a receptor for VacA (33). Increased binding of acid- or alkali-activated VacA to RPTP β may alter its activity and possibly accelerate or inhibit the dephosphorylation of tyrosine on cytosolic proteins. Further study of the function of RPTP β in target cells may well provide further information concerning the mechanism of VacA toxicity on host cells.

4. Vacuolation and cell death

The membranes of VacA-induced vacuoles are intermediate compartments between late endosomes and lysosomes; the vacuoles contain both a late endosomal marker, the small GTP-binding protein Rab7, and a lysosomal marker, the membrane protein Lgp 110 (34). Accordingly, VacA might disrupt normal membrane trafficking at or near the level of late endosomes. The microinjection of VacA or the transfection of plasmids containing the VacA gene into HeLa cells resulted in the formation of intracellular vacuoles (35), providing evidence that VacA introduced into the cytosol acts on an intracellular target, and represents an important advance in understanding the mechanism of VacA action. With regard to the intracellular target of VacA, VIP54 has been identified as a cytosolic VacA-binding protein by yeast two-hybrid screening of a HeLa cell library (36). Rac1, a small GTP-binding protein, which regulates remodeling of the actin cytoskeleton, and dynamin, a high molecular weight G-protein, which is assumed to function at an early step of vesicle formation during endocytosis, also play an essential role in VacA-induced vacuolation (Fig. 4) (37,38). Although the mechanism by which VacA enters cells is not yet defined, it is possible that the 58-kDa fragment of VacA and RPTP β have a role.

VacA induced an inhibition of energy metabolism followed by mitochondrial damage, leading to impairment of the cell division cycle in AZ-521 cells (39,40). VacA and its 34-kDa fragment induce cytochrome c release and the cleavage of poly(ADP-ribose)polymerase, leading to HeLa cell death by

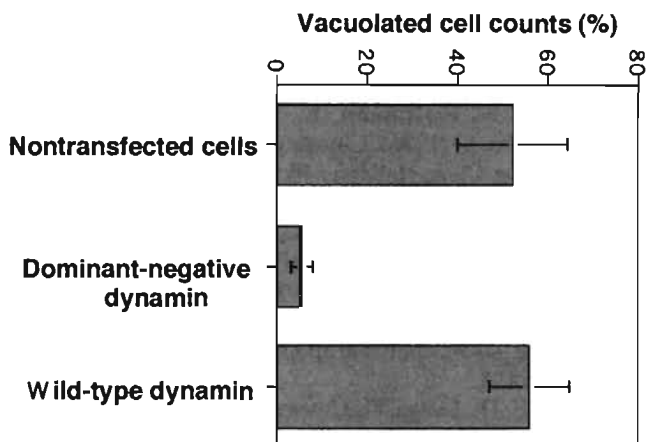


Fig. 4. Numbers of VacA-vacuolating cells in HeLa cells transiently transfected with dominant-negative dynamin-1 or wild-type, and nontransfected HeLa cells. Results are expressed as percent of vacuolated cells (38).

apoptosis (41). VacA might be at least one factor causing cell death by apoptosis observed in *H. pylori* infection (42).

5. Conclusion

H. pylori produces and secretes a potent cytotoxin, VacA, which induces cytoplasmic vacuolation and cell destruction, leading finally to gastric injury. Our recent report suggests that VacA interacts with RPTP β -associated signal transduction pathways, which are essential for toxicity. Although Ogura et al. reported that Mongolian gerbils challenged by VacA mutant strain induced severe gastritis (43), further understanding of the pathological responses of wild type and RPTP β -deficient animal models may well provide valuable information regarding the mechanism of VacA toxicity, that is, whether RPTP β is essential for the induction of gastric ulcers by VacA.

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