Protein tyrosine phosphatase β, a receptor for Helicobacter pylori VacA toxin

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Abstract. 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. 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. A 250-kDa receptor protein tyrosine phosphatase (RPTP) β served as a receptor for VacA on AZ-521 cells. 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. Increased binding of acid- or alkali-activated VacA to RPTP β may alter its activity and possibly accelerate or inhibit dephosphorylation of tyrosine on cytosolic proteins. Understanding the pathological responses of wild type and RPTP β-deficient animal models may well provide valuable information regarding the mechanism of VacA toxicity.

Key words: Vacuolating cytotoxin, VacA, toxin receptor, Helicobacter pylori, protein tyrosine phosphatase β

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 the 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. H. pylori infection is responsible for human diseases, is mostly acquired in childhood and persists chronically when untreated. The persistent H. pylori infection results in the induction of an inflammatory response and epithelial cell 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 potent protein toxin, the vacuolating cytotoxin, VacA, which induces cytoplasmic vacuolation and apoptosis, leading to gastric injury. Before VacA purification, an immunodominant protein encoded in the cagA gene, which is located on the border of a pathogenicity island of H. pylori, was mistaken for VacA. Although recent work showed that the CagA, which is injected by its type IV secretion system and tyrosine phosphorylated in host cells,9-11 forms a complex with SHP-2 tyrosine phosphatase,12 the function of CagA is still unknown. Type-I strains, which produce both VacA and CagA, are considered more pathogenic and are more likely than the type-II strains lacking VacA activity and the pathogenicity island to be associated with the severe diseases (e.g., ulceration, adenocarcinoma).13 Oral administration of VacA causes gastric mucosal damage in mice14-17 and VacA has been proven epidemiologically to be a virulence factor associated with peptic ulcer in humans.18,19

Structure and Biological Activity of VacA

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

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*. 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.

VacA of the m1 genotype is an oligomeric toxin, composed 5 or 6 subunits. The monomer of VacA sometimes is 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 toxins. 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.

**Activation of VacA by Alkaline or Acid Conditions Increases Its Binding to a 250-kDa-glycoprotein Receptor, Tyrosine Phosphatase (RPTP) β**

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*. In addition, VacA was activated, not only under acidic, but also alkaline conditions resulting in its enhanced binding to target cells. Although the detailed mechanism is the subject of much speculation, taken together with the recent report on tertiary structure of VacA, the acid- and alkalai-activation of VacA might reflect a molecular change from hexamer to heptamer, resulting in exposure of some portion of the protein surface needed for receptor binding. *H. pylori* persistently grows in the mucus layer of the gastric tissue by means of a potent urease that produces ammonia; this product plays a significant role in the neutralization of the acidic environment of the stomach and allows the bacteria to adhere to the apical plasma membrane of surface epithelial cells in the pyloric antrum in *vivo*. In *vivo* *H. pylori* adhered to AGS human gastric adenocarcinoma cells; *H. pylori* appeared to be partially engulfed by the plasma membrane which was associated with a loosely organized network of actin filaments. It is probable that a non-acidic or alkaline surface environment is formed by ammonia produced from urea secreted by gastric epithelial cells by action of *H. pylori* urease, even though gastric juxtamucosal pH of patients infected with *H. pylori* is 5.7.

Analysis of the proteolysis patterns indicated that neutralization of the alkaline (pH 11.5)-treated VacA induces a transition to a conformational state which is similar to that of acid (pH 1.5)-treated VacA and different from the untreated toxin. After digestion of acid- and alkali-treated VacA with trypsin and proteinase K, 50- and 30 kDa proteins were observed by SDS-PAGE. These products were absent when untreated VacA was used. Velocity sedimentation analysis showed that treatment of VacA at alkaline pH led to disassembly of VacA oligomers as reported for acid-treated VacA.

Rapid vacuolation of AZ-521 cells incubated with acid- and alkaline-treated VacA was associated with the enhanced binding to a 250-kDa surface glycoprotein, termed p250, having galactose-β(1-3)-N-acetylgalactosamine and galactose-β(1-4)-N-acetylgalactosamine. These data suggest that acidic and alkaline treatments induce a conformational change in the VacA molecular structure that leads to its activation and consequently increased binding p250 on target cells.

p250, purified by chromatography on peanut agglutinin (PNA)-affinity and Superose 6 columns, contained N-terminal and internal amino acid sequences of YRQQRKLVVEIGWST and LIIQDIELATQDY, respectively. These sequences are identical to those of a receptor protein tyrosine phosphatase (RPTP β/PTP ξ). In agreement, p250 reacted with anti-human RPTP β monoclonal antibody. Immuno-precipitation with anti-human RPTP β antibody of solubilized membrane preparations previously incubated with VacA or heat-inactivated VacA demonstrated that RPTP β bound native, but not denatured VacA. The data also suggested that acidic and alkaline treatments induce a molecular change in VacA that is associated with its activation and increased binding to cell surface RPTP β.

**Morphologic Differentiation of HL-60 Cells is Associated with Appearance of RPTP β and Induction of VacA Sensitivity**

Phorbolmyristate (PMA, TPA; 12-O-tetradecanoyl phorbol 13-acetate) induces differentiation of the human leukemic cell line HL-60 into cells with macrophage-like characteristics and enhances the susceptibility of HL-60 cells to the Helicobacter pylori VacA toxin. We examined the mechanism by which HL-60 cells acquire sensitivity to VacA, in particular, looking for expression of RPTP β, a VacA-binding protein postulated to be the VacA receptor.
induced expression of RPTP β mRNA and protein as determined by RNase protection assay and indirect immunofluorescence studies. Vitamin D3 and IFN-γ, which stimulate differentiation of HL-60 cells into a monocyte-like cells, also induced VacA sensitivity and expression of RPTP β mRNA, whereas 1.2% DMSO and retinoic acid, which stimulated the maturation of HL-60 into granulocyte-like cells did not. RPTP β antisense oligonucleotide inhibited induction of VacA sensitivity and expression of RPTP β. Double immunostaining studies also indicated that newly expressed RPTP β colocalized with VacA in PMA-treated HL-60 cells. BHK-21 cells transfected with the RPTP β cDNA acquired VacA sensitivity. All data described here are consistent with the conclusion that acquisition of VacA sensitivity by PMA-treated HL-60 cells results from induction of RPTP β, a protein that functions as the VacA receptor.38

Vacuolation and Cell Death by VacA

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 110.39 According to 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.40 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 was identified as a cytosolic VacA-binding protein by yeast two-hybrid screening of a HeLa cell library.41

VacA induced an inhibition of energy metabolism followed by mitochondrial damage, leading to impairment of the cell division cycle in AZ521 cell.42,43 VacA and its 34 kDa fragment induce cytochrome c release and the cleavage of poly(ADP-ribose)polymerase, leading to HeLa cell death by apoptosis.46 VacA might be at least one factor causing cell death by apoptosis observed in H. pylori infection.47 Although the exact mechanism by which vacuolization and apoptosis are related to signaling from RPTP β and VacA remains to be determined, we believe that further study of the function of RPTP β in target cells may well provide valuable information concerning the mechanism of VacA toxicity. In addition, it is notable in H. pylori infection that both VacA- and CagA-induced cell damage are mediated via perturbation of tyrosine phosphorylation in host cells.

References