Chapter 5

VacA Genotype in *Helicobacter pylori*

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Abstract

Helicobacter pylori infection has been recognized as a worldwide problem. *H. pylori* infection is the most prevalent cause of chronic gastritis and has been related to peptic ulcer disease and gastric cancer. It is considered that *H. pylori* infects half of the world's population. Several virulence factors are produced by *H. pylori* in which each of them is related to an increase in the risk of disease development. The vacuolating cytotoxin (VacA) is one of these virulence factors. The first defined action of VacA was induction of intracellular vacuolation. VacA uses a variation in other effects on target cells, such as disruption of mitochondrial functions, stimulation of apoptosis, and blockade of T-cell proliferation, for the induction of vacuolation. In addition, VacA has an important role for colonization of *H. pylori* in vivo.

Keywords: *Helicobacter pylori*, disease, vacuolating cytotoxin (VacA), vacuolation

1. Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative and microaerophilic bacterium, which usually colonizes in the human stomach. *H. pylori* affects about half of the human population worldwide, which exists in their upper gastrointestinal tract. Though all the factors have not been known, we could say that the infection is most likely to happen at a young age and happens more common in developing countries [1]. Prevalence of infection is through human contact mainly via the gastric-oral way [2]. *H. pylori* is related to some diseases such as peptic ulcer disease, gastric ulcers, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer [3].

Study on this microaerophilic spiral-shaped bacterium is interesting. *H. pylori* is a part of a quickly growing genus. New species are being derived from numerous vertebrate hosts.

In addition, other Helicobacter species are being derived from nongastric parts in humans and might have a role in diseases that formerly had no certain etiologic factor.

H. pylori has polar-sheathed flagella, which helps in motility. In addition, these structures also have a terminal bulb that could make it more adapted to swimming through mucus. Moreover, on the surface, there are special biological characteristics in the lipopolysaccharide, and in order to escape from the host responses, genes that control addition of the O-side chains can phase vary. Moreover, *H. pylori* has a special peptidoglycan structure, which is different from other gram-negative bacteria. Also *H. pylori* releases an autotransported vacuolating cytotoxin that makes the abnormal phenotype of vacuolation in host cells.

For the first time in 1982, two Australian scientists Barry Marshall and Robin Warren identified *H. pylori* in a patient with chronic gastritis and gastric ulcers. Before that, it was not believed to have a microbial reason. By the successful culture of *H. pylori*, a large number of researchers investigated the epidemiology of transmission of the organism. In addition, it is connected with the development of duodenal ulcers and stomach cancer. More than 80% of infected population with the bacterium are asymptomatic, and it might have an important impress in the natural stomach ecology [4]. However, almost 10–15% of people infected with *H. pylori* shown severe gastric disorders containing peptic ulcers, gastric lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma [5].

In 1983, *H. pylori* was cultured from human gastric tissue for the first time [5]. After many years, a proteinaceous component known as "vacuolating cytotoxin" was found in *H. pylori* broth culture supernatants. By adding vacuolating cytotoxin to cultured eukaryotic cells, the cells became vacuolated [6]. Formerly, bacterial toxins had not been reported by this function. In the further studies, the identity of the vacuolating toxin was shown [7, 8] and revealed that vacuolating cytotoxin (VacA) has different characteristics and activities compared to other bacterial toxins.

Multiple virulence factors are produced by *H. pylori* in which each of them is related to an increase in the risk of disease extension. Cytotoxin-associated gene A (CagA) and the vacuolating cytotoxin (VacA) are the virulence factors [9].

Infection by *H. pylori* strains including the toxigenic allelic s1 form of VacA increased the risk of peptic ulceration and gastric cancer [10]. VacA was termed because of its ability to cause "vacuole"-like membrane vesicles in the cytoplasm of gastric cells [11]. However, its function in *H. pylori* pathogenesis has not been clear yet. VacA is a pore-forming toxin (PFT). VacA uses a variety of other effects on target cells, such as disruption of mitochondrial functions, stimulation of apoptosis, and blockade of T-cell proliferation, for the induction of vacuolation [12]. In addition, VacA has an important role for colonization of *H. pylori* in vivo [13].

A type IV secretion system is encoded in the cag pathogenicity island (cagPAI), and it replaces CagA into gastric epithelial cells. It causes morphological changes and proinflammatory cytokine secretion [14].

2. *H. pylori*

The gastric mucosa of almost 50% of the world's population has colonized by *Helicobacter pylori* and is related *to gastroduodenal diseases ranging from superficial and chronic gastritis,

and duodenal and gastric ulcers to gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. [15–17]. There is also some evidence that infection by *H. pylori* may be related in increasing the intensity or risk of infection by other gastrointestinal pathogens and in childhood malnutrition, especially in countries that are less developed [18, 19].

H. pylori was the first bacterial species that is genome sequenced and compared with two independent isolates [20, 21]. Further comparison has presented the first detailed view point at the physical chromosomal organization and has started to recognize a minimal set of common genes that can be considered as candidates for therapeutic strategies.

The two independent *H. pylori* genomes, which are completely sequenced, have different origins. *H. pylori* 26695 was isolated from a patient with gastritis in the United Kingdom in the early 1980s and sequenced by the Institute for Genomic Research [22]. Before sequencing, this strain has been passaged frequently in the laboratory. Also, *H. pylori* J99 was isolated from a patient with a duodenal ulcer and duodenitis in the United States in 1994 and sequenced in a collaborative effort between Astra AB (now AstraZeneca PLC) and Genome Therapeutics Corporation. This strain had not been extensively passaged before sequencing [20]. By using a random shot-gun approach from libraries of cloned chromosomal fragments of ~2.5 kb, J99 and 26695 were sequenced. Like the most microbial genome sequencing projects until now about 45,000 sequence reads in the case of J99 using PHRAP, which resulted in 68 nonredundant contigs, representing almost 98% of the genome, assembled it. PFGE analysis and probe hybridization confirmed the assembly of the *H. pylori* J99 genome [20].

The 26695 genome was 24 kb larger than J99. However, both the J99 and 26695 genomes possessed a total (G + C)% of 39%. There is some similarity in J99 and 26695, such as average lengths of coding sequences, coding density, and the bias of initiation codons. The genome of J99, consistent with the genome of strain 26695, had no clearly recognizable origin of replication. Near the origin of replication in prokaryotes, specific genes, including dnaA, dnaN, and gyrA, are often detected. However, these genes are not in close nearness either to each other or to the repeated heptamer that was determined as nucleotide number one in both published *H. pylori* sequences.

Additional evidence is that this position may be regarded as the replication origin achieved from using an algorithm that analyzes the bias of short oligomers whose direction is preferentially skewed around the replication origin of prokaryotes [23].

Leunk et al. found massive vacuolar degeneration of various cultured epithelial cell lines in supernatants from broth cultures of *Helicobacter pylori*, in 1988 [6]. After that, numerous studies throughout the world have done on the nature of this toxic activity and its effect in *H. pylori*-induced disease. The protein mediating the effect was purified and called the vacuolating cytotoxin in 1992 [7]. In 1994, discovery of the amino-terminal sequence of the protein led to the cloning and sequencing of the toxin gene, which was nominated vacA [8, 24–26].

Subsequent to the primary characterization of the toxin and its gene, research has focused on VacA structure, the mechanisms underlying VacA's toxic activity, naturally happening differences between VacA proteins produced by various strains of *H. pylori*, and the clinical significance of VacA polymorphism.

Studies on VacA has expanded, not only because of its potential as a novel tool for exploring features of eukaryotic cell biology but also mainly because of its supposed function in

the pathogenesis of *H. pylori*-related diseases, in specific peptic ulceration and distal gastric adenocarcinoma. The accurate function of VacA in these diseases is still under research, but VacA may contribute to the capacity of *H. pylori* to colonize and persist in the human gastric mucosa and may also contribute immediately to gastric epithelial damage. Therefore, VacA is a purpose for therapeutic intervention and a candidate for inclusion in a vaccine against *H. pylori*.

3. The *vacA* gene

Vacuolating cytotoxin (vacA) is the most commonly identified virulence factor among *H. pylori* strains. VacA belongs to the group of genes with mutable genotypes related to damage to gastric epithelial cells. This gene exists in almost all strains of *H. pylori*. This gene is polymorphic and contains variable signal regions (type s1 or type s2) and midregions (type m1 or type m2) [27] and intermediate regions (i1 and i2 alleles, and the rare i3 allele) [28] (**Figure 1**). There are various levels of its cytotoxicity that is caused by the variety of signal (s) and mid (m) regions of vacA gene [29]. S region variations are more related to the vacuolating activity of vacA, and m region variations have effect on binding of the toxin to the host cells, as reasons are contributed to define cell specificity [29].

A copy of the toxin gene, vacA, exists in all *H. pylori* strains. The vacA transcript is monocistronic. Transcriptional start point in this gene is located about 119 nucleotides upstream from the ATG start codon [25, 30]. The capacity of *H. pylori* to induce vacuolation in epithelial cells abrogates by insertional mutagenesis of vacA. In addition, it interrupts a number of other vacA-induced toxic effects [24, 25, 31]. Alleles of vacA from about 25 different *H. pylori* strains have been sequenced and range from 3864 to 3933 nucleotides in length [8, 24, 32–34].

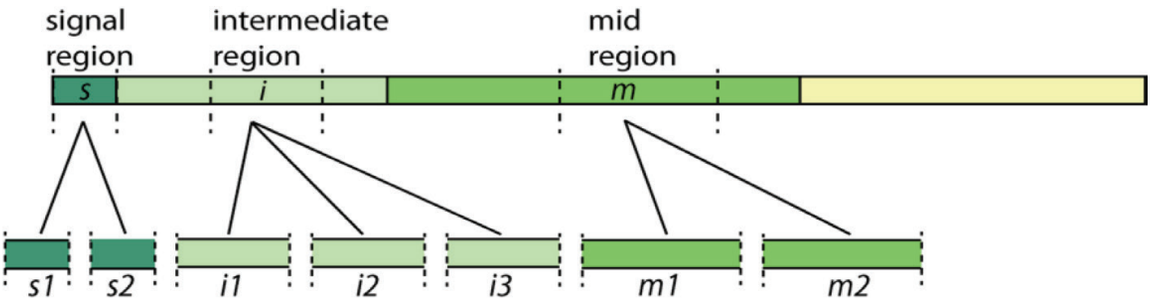

Figure 1. VacA gene.

4. The association of vacA types with cytotoxin production

Significant genetic diversity exists between vacA alleles from different strains. These alleles could be classified into various families. The most significant studied form of VacA is encoded

by type s1/m1 vacA alleles. s1/m1 vacA alleles typically encode VacA proteins, related to a high level of vacuolating cytotoxin activity [32], while s1/m2 strains have moderate toxin production [29]. In fact, s1/m2 strains that have an i1 allele are able to induce vacuolation. However, s1/m2 strains that have an i2 allele are not able to induce vacuolation [28]. s2/m2 strains have rare or even absent toxin production [29].

By comparing vacA s1 and m1 strains with vacA s2 and m2 strains, which are less virulent, it was revealed that *H. pylori* vacA s1 and m1 strains are related to higher levels of inflammation in the gastric mucosa and increased risk for gastric atrophy and carcinoma. After the explanation of the vacA i-region, it was also revealed that the determinant of cytotoxicity i1 allele is related to gastric carcinoma [28].

5. VacA proteins

VacA encodes a protein with a mass of about 140 kDa; however, under denaturing conditions, the mature secreted VacA toxin drifts as a band of almost 90 kDa [7, 8, 24–26]. A comparison of the amino-terminal sequence of the mature secreted toxin with that predicted for the protoxin shows that a 33-amino-acid amino-terminal signal sequence is cleaved during the procedure of VacA secretion. Investigations by antisera raised against various regions of recombinant VacA show that a polypeptide of about 33 kDa isolated from the carboxy-terminal portion of the protoxin stays localized to the bacteria and is not secreted [26]. This carboxy-terminal portion of VacA seems to contain amphipathic β-sheets capable of forming a β-barrel structure and has a terminal phenylalanine-containing motif that is available in several outer membrane proteins [25]. These qualities, with a pair of cysteine residues nearly the carboxy-terminus of the mature secreted protein, are specification of a family of secreted bacterial proteins called autotransporters [35]. Autotransporters for export across the bacterial outer membrane do not need any auxiliary proteins. By studying the Neisseria gonorrhoeae IgA1 protease, we achieved this information of autotransporter export. Translocation of IgA1 protease through the bacterial cytoplasmic membrane is achieved via a Sec-mediated process and is accompanied by cleavage of an amino-terminal signal peptide. After inserting the carboxy-terminal β-barrel domain into the outer membrane, it functions as a pore through that the residue of the molecule passes. The mature secreted IgA1 protease is produced by autoproteolytic cleavage. The carboxy-terminal domain stays related to the outer membrane [36].

Primary studies showed, despite mature VacA monomers are about 90 kDa in mass, that the toxin exists as a much larger complex or aggregate under nondenaturing conditions [7]. Lupetti et al. investigated the ultrastructure of purified VacA using deep-etch electron microscopy. They illustrated that the toxin forms into large flower-shaped complexes that appear to consist of a central ring surrounded by six or seven "petals" [37]. An accurate view of the surface of VacA oligomers is presented by three-dimensional reconstructions of these deep-etch metal copies (**Figure 2**) [38]. Moreover, the classical flower-like complexes, VacA, could be assembled into other type of complex, that is named a "flat form," which includes of six or seven petals without a notable central ring [37, 39].

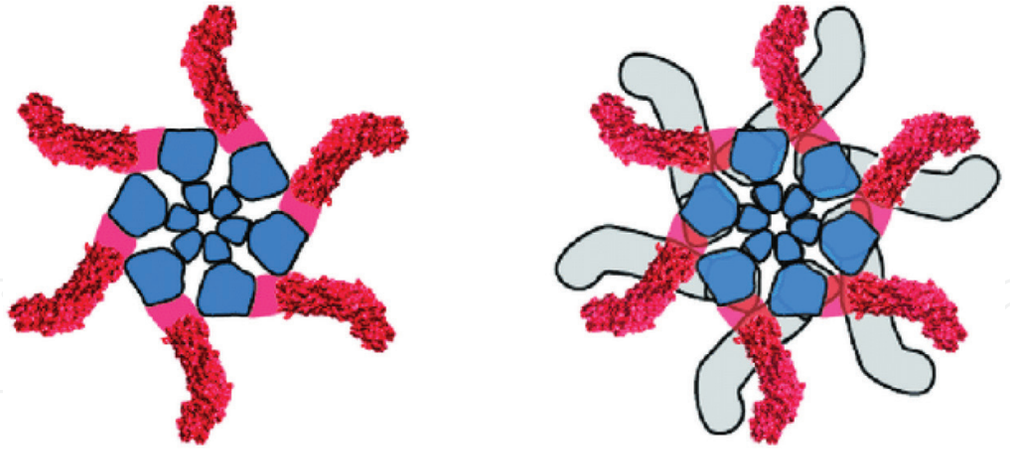

Figure 2. VacA oligomer.

The petals that contain the flat form generally radiate from the center of the complex with a specific clockwise chirality. Several models have suggested clarifying the assembly of VacA into flower-like complexes and chiral flat forms. In one of these models, the flower-like forms are proposed to contain six or seven monomers of about 90 kDa [38, 39]. In another model, the flower-like forms are considered to be dodecamers or tetradecamers of VacA monomers of about 90 kDa, and flat forms are proposed to be hexamers or heptamers [37]. In acidic or alkaline pH, VacA oligomers separate into monomeric parts of approximately 90 kDa, each measuring of about 6 by 14 nm [37, 40, 41]. This pH-mediated disassembly is related by a marked enhance in VacA cytotoxic activity [39, 41–43].

This opinion exists that VacA monomers have more cytotoxic activity than water-soluble VacA oligomers. Subsequent researches about VacA structure have been undertaken using atomic force microscopic imaging of purified toxin bound to supported lipid bilayers [44].

Two-dimensional crystalline arrays of VacA on lipid bilayers include an arranged array of hexagonal central rings connected by thin connectors to peripheral domains.

In-frame deletions in the portion of VacA encoding the amino-terminal region of the toxin produced mutant strains of *H. pylori*. Mutant strains of *H. pylori* express truncated VacA proteins. These proteins are secreted, though fail to oligomerize and lack recognizable cytotoxic activity [45, 46]. VacA Δ91–330 is a mutant VacA protein that has water-soluble dimeric form, which has an ultrastructural appearance similar to that of the peripheral petals of VacA oligomers [45]. The peripheral petals of VacA oligomers can be consistent with the carboxy-terminal portion of the mature secreted VacA polypeptide.

6. Functional domains in VacA

The purified ~90 kDa VacA toxin through extended storage or incubation with trypsin break down into ~37 and ~58 kDa components, which are isolated from the amino terminus and

carboxy terminus of the protein. Proteolytic cleavage occurs at a site containing multiple charged amino acids [26]. In fact, the 37 and 58 kDa fragments of VacA are considered as subunits or domains of the holotoxin [47].

Burroni et al. manufactured an *H. pylori* mutant in order to specify whether cleavage of VacA into 37 and 58 kDa fragments is needed for toxin activity. In this *H. pylori* mutant, the region of vacA encoding the 46 amino acids flanking the VacA cleavage site was removed [48]. Because of this fact that this mutant VacA was entirely active, it is informing that cleavage of the exposed loop is not required for activity. While the wild-type VacA produced by the parent strain prefer to form seven-sided complexes, in contrast, the mutant prefers to form six-sided complexes. This revealed that deleting the exposed loop presented structural restriction.

In experiments wherever mutant forms of vacA under the control of a eukaryotic promoter have been expressed from plasmids in the cytosol of epithelial cells, it has been explained that the minimal region of VacA is required for vacuolating activity [11]. These experiments revealed that the epithelial cell lines, which transfected with plasmid constructs encoding either the full-length ~90 kDa secreted toxin or amino- or carboxy-terminally truncated fragments. In addition, these experiments represented that a VacA protein lacking most of the carboxy-terminal 58 kDa domain preserved complete vacuolating activity [49, 50]. By eliminating 10 amino acids from the amino-terminus, activity was entirely abolished, and by eliminating 6 amino acids from the amino-terminus, activity was only in part abolished [49, 50]. The minimal VacA domain that presented complete vacuolating activity when expressed intracellularly was a peptide containing amino acids 1–422, which is the 37-kDa domain plus a fragment of the 58-kDa domain [50]. The 37 kDa fragment was inactive in alone, but coexpression of this fragment with a fragment including the amino-terminal 165 amino acids of the 58 kDa fragment resulted in complete vacuolating activity [50]. A conceivable explanation for the importance of the VacA amino terminus was determined by hydrophobicity plots. In fact, the only hydrophobic region in VacA is amino acids 1–32 of this region, and it is long enough to span a membrane.

A *H. pylori* vacA partial deletion mutant was produced, which lacked codons for amino acids 6–27, for more research [51]. The structure of mutant VacA did not have variations compared to wild-type VacA; however, mutant VacA lacked cytotoxic activity.

In addition, alanine scanning mutagenesis showed that point mutations at proline 9 or glycine 14 entirely abrogated VacA activity [52]. Another factor that abrogated toxin activity is the addition of an amino-terminal hydrophilic extension to VacA [53]. As a result, it is obvious that the amino-terminal hydrophobic region has an important role in toxin activity.

7. Receptor binding region

It is demonstrated that amino acid sequences located in the carboxy-terminal portion of the mature protein mediated binding of VacA to cells.

Investigations on the purified 58 kDa fragment from a mutant *H. pylori* strain represent that this protein binds to HeLa cells with kinetics similar to those of the intact toxin [45]. The binding of VacA to cells is inhibited by polyclonal antiserum reactive with the 58 kDa domain [54]. Several natural forms of VacA have significant divergent amino acid sequences in the 58 kDa domain, which are called m2 forms. These forms cause vacuolation in a more confined range of cultured epithelial cell lines. Differences in cell binding would be a reason for this [34]. VacA with a type m2 58 kDa domain, that did not cause HeLa cell vacuolation when applied externally, affected vacuolation when expressed from a plasmid in the HeLa cell cytoplasm. This indicates that m2 VacA is entirely active but cannot get to its site of action. This would be because of inability to bind to the cell [11].

Investigations by naturally occurring and engineered m1/m2 chimeric proteins [55] propose that an ~40 amino acid region near the amino-terminal end of the 58 kDa domain is required for HeLa cell vacuolation and can have a role in HeLa cell binding.

8. Activity of VacA

Epithelial cell vacuolation in vitro occurs by VacA; however, this does not cause cell death quickly. Cell death in human gastric epithelial cells that are exposed to high doses of toxin is reported after 2 days [56]. On the other hand, cell death does not normally happen in immortalized cell lines exposed to the toxin. As an example, incubation of AZ-521 gastric epithelial cells with VacA for several hours causes decreased mitochondrial ATP production and decreased oxygen utilization but does not result in cell death [57].

The exact mechanisms of binding and uptake of VacA by cells are not clearly understood yet. The prototypic s1/m1 form of VacA binds to HeLa cells in a saturable manner recognized by flow-cytometry analysis [58]. However, saturable binding has not been indicated with classical ligand binding assays with 12I-labeled VacA [43]. Activation of VacA by acid treatment significantly increases its vacuolating activity but does not remarkably increase its binding to HeLa or Baby Hamster Kidney (BHK) cells [40, 58]. However, binding of the toxin to the gastric cell line AZ-521 is increased by acid activation [41]. A number of specific VacA receptors were proposed. Activated VacA binds to a 250 kDa receptor protein-tyrosine phosphatase β (RPTPβ) in the AZ-521 system that regulates intracellular tyrosine phosphorylation [41, 59].

Autotransporters are a family of secreted bacterial proteins, which are determined by mentioned features, together with a pair of cysteine residues near the carboxy-terminus of the mature secreted protein. It is suggested that RPTPβ has an important role in binding VacA to cells and following intoxication. Treatment of the HL-60 cell line with phorbol 12-myristate 13-acetate (PMA) causes stimulation of RPTPβ expression that is occurred with stimulation of VacA sensitivity [60]. BHK-21 cells are insensitive to VacA, but transfection with expression vectors including the RPTPβ gene can make them sensitive. Antisense oligonucleotides in PMA-treated HL-60 cells lead to ablation of RPTPβ synthesis. As a result, a considerable reduction occurs in VacA-induced vacuolation. An unidentified 140 kDa protein in AZ-521 and AGS cells and the epidermal growth factor receptor in HeLa cells [61, 62] are two other particular VacA receptors. These evidence suggested that multiple surface-binding sites

recognized by both inactive and activated VacA exist; in addition, specific VacA receptors exist that are variably expressed in different cell lines.

Both 58 and 37 kDa regions are needed for VacA internalization [45]. VacA should be preactivated by disposal of acid or alkali, in order to be internalized [43]. Internalization happens through an energy-dependent process; the exact nature of which is not clear. However, it may be a receptor-mediated endocytosis. VacA molecules localize in membrane vesicles, after internalization [54]. Then localized VacA molecules are transported along the endocytic pathway to vacuolar-type (V-) ATPase-positive late endosomes and lysosomes. In this state, they accumulate and persist for some days [63, 64].

The first defined action of VacA was induction of intracellular vacuoles [64, 65]. The vacuolar membranes include both late endosomal and lysosomal markers, indicating that the vacuoles are derived from these sections [66, 67].

The complete activity of V-ATPase and the existence of weak bases are needed for the formation of VacA-induced vacuoles, which indicated that vacuoles are derived from the accumulation of weak bases within acidic sections, and with water influx and swelling followed [63, 64, 68, 69]. Moreover, the membrane traffic regulator rab7 and the actin-cytoskeleton-associated Rac1 are two small GTP-binding proteins that involved in vacuole biogenesis [70, 71]. Rac1 and rab7 are related with the membrane of VacA-induced vacuoles. The expression of rab7 or Rac1 dominant negative mutants inhibits vacuolization, and the expression of rab7 or Rac1 dominant positive mutants potentiates vacuolization. It has been proposed that membrane fusion events and the cytoskeleton supporting late endosomal sections regulated vacuole development. VacA destructs the transport of acidic hydrolases to lysosomes and causes the release of these enzymes into the extracellular medium in HeLa cells [72]. VacA caused decrement of the degradative power of HeLa cell lysosomes and also decrement of the antigen-processing compartment of B lymphocytes [72, 73].

VacA is unable to vacuolate epithelial monolayers of MDCK I, T84, or epH4 cells on porous filters. In addition, MDCK I, T84, or epH4 cells do not show signs of endolysosomal dysfunction [74].

Subsequently, disposal to VacA, transepithelial electrical resistance (TER) reduces, occurred with an increase in transepithelial flux of low-molecular-weight molecules [74]. There are some reasons, which propose that VacA modulates the resistance of these model epithelia through a paracellular effect. These reasons include the size selectivity of this increased epithelial permeation, lack of accompanying vacuolation, and lack of redistribution of junctional proteins. Just epithelial cell monolayers capable of expanding a TER higher than 1000–1200 Ω/cm^2 are affected. By utilizing the isogenic mutant strains, this is confirmed that the effect is dependent on VacA [31]. In MDCK cells, m2 type of VacA decreases TER. However, it does not lead to vacuolation in this cell line even when cells are nonconfluent [31]. It is corroborated that vacuolation and increased permeability of monolayers are separate and independent effects.

VacA constructs ion channels in model lipid bilayers and cell plasma membranes. This occurrence may underlie all the other consequences of VacA. Acidic conditions cause disassembly of the inactive VacA oligomer, which permits insertion of the toxin into lipid bilayers [66, 73, 75].

Investigations with planar model membranes represent that membrane insertion is followed by the formation of voltage-dependent, low-conductance (10–30 pS in 2 M KCl), and anion-selective channels [76, 77].

Patch clamp analysis of HeLa cells indicates that VacA forms plasma membrane channels with features similar to those perceived in model membranes [78]. Different anion channel blockers inhibit VacA channels in vitro with various powers and are able to prevent and partially inverse vacuolation of HeLa cells [78, 79], informing an essential role of the anion channel in vacuolation [41]. With permitting anions to permeate into late endosomes, the endocytosed VacA channel increases the turnover of the electrogenic V-ATPase that causes accumulation of weak bases and leads to vacuole formation by water influx [80, 81]. Because of that, internalization of surface-bound VacA is required for the further development of vacuolation; this hypothesis is acceptable [43]. Vacuolation in this model can be considered as a side effect of the massive accumulation of endocytosed VacA channels in endolysosomes. With 5-nitro-2-(3-phenylpropylamine) benzoic acid (NPPB), VacA epithelial permeabilization of MDCK I cells can be partly prevented and reversed, the most efficient blocker of VacA channels, implying that epithelial permeabilization, similar to vacuolation, is less important for the formation of apical anion channels [78]. VacA induces an increased apical anion secretion in Caco-2 cells, and this also is blocked by NPPB [82], implying that it is also because of VacA anion channel formation.

9. Discussion

H. pylori is considered as a significant cause of chronic active gastritis, peptic ulcer, and atrophic gastritis. It is related to an enhanced risk of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT). VacA is a virulence factor related to peptic ulcer. Moreover, oral administration of VacA leads to gastric mucosal damage in mice. It could be concluded that VacA might contribute to epithelial cell damage or peptic ulceration in H. pylori-infected humans [83].

A toxin that has damaging outcomes on epithelial cells is produced by *H. pylori*. In addition, colonization of *H. pylori* has contributed in the development of peptic ulceration. By this information, it could be concluded that VacA directly harms the gastric and duodenal epithelium in vivo; therefore, it leads to ulcers. *H. pylori* strains which have vacuolating cytotoxin activity in vitro are more often associated with disease than *H. pylori* strains which are noncytotoxic strains. There is a significant association between vacuolating activity and peptic ulcer disease, which is revealed by some studies all over the world [32, 84–86]; however, this association is not true in all situations. Often noncytotoxic *H. pylori* isolates from patients with peptic ulceration, and cytotoxic *H. pylori* isolates from patients without peptic ulceration. However, explanation of these studies depends on some factors.

There have been many studies on the relationship between specific vacA genotypes and diseases, which are developed by multiple vacA genotypes and explained by polymerase chain reaction (PCR)-based methodology for discrimination between them [32, 81, 87]. It was

proved by most of these studies from outside Asia that s1 strains are more often associated with peptic ulceration or gastric carcinoma than s2 strains [32, 81, 88–91].

H. pylori vacA s1 and m1 strains are related to higher levels of inflammation in the gastric mucosa and increased risk for gastric atrophy and carcinoma. In addition, it was revealed that the determinant of cytotoxicity i1 allele is related to gastric carcinoma. So, evaluation of characterization of this region as a determinant of the clinical outcome of *H. pylori* infection could be used [28].

10. Conclusion

Helicobacter pylori has been investigated since its first culture in 1982 from a gastric biopsy. Cytotoxin-associated gene A (CagA) and the vacuolating cytotoxin (VacA) are the virulence factors which are produced by *H. pylori* and are related to an increase in the risk of disease extension [9].

VacA has a significant role in the pathogenesis of *H. pylori*-associated diseases, especially in peptic ulceration and distal gastric adenocarcinoma. For this reason, VacA has been studied widely. VacA is still under examination in order to find out its accurate role in these diseases. However, VacA makes *H. pylori* able to colonize in the human gastric mucosa. Moreover, VacA could have a role in gastric epithelial damage. For this reason, VacA is a target for therapeutic intervention and also is considered for usage in a vaccine against *H. pylori*.

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