

COULD KNOWLEDGE OF *H. PYLORI* PATHOGENICITY FACTORS LEAD TO THE EMERGENCE OF NEW METHODS FOR IDENTIFYING BACTERIA?

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Abstract: *H. pylori* is an extracellular bacterial pathogen adapted to colonizing the gastric mucosa. It is currently recognized as the most common etiological factor involved in the genesis of digestive disorders with localization in the gastric mucosa, present in two thirds of the world population in relation to socio-economic status. The relationship between the *H. pylori* bacteria and the human host can be explained in three stages, each involving specific factors. In the first stage *H. pylori* crosses the stomach and enters in the gastric mucosa. The next step of joining the gastric epithelium and colonization of the mucosa leads to long-term resistance to treatment and chronic infection and it is facilitated by the genotypic and phenotypic diversity of *H. pylori*. In the third stage we can observe the harmful action on the gastric mucosa.

Key words: *H. pylori* pathogenicity, new methods of identifying bacteria.

1. Introduction

H. pylori is an extracellular bacterial pathogen adapted to long-term colonization of the gastric mucosa. Currently it is recognized as the most common etiologic factor involved in the genesis of digestive disorders with localization in the gastric mucosa, being present at two thirds of the world population in accordance to socio-economic status.

Clinical manifestations of *H. pylori* infection may even occur outside the

digestive system, as antigenic similarity between *H. pylori* antigens and the patient's antigens can cause autoimmune disorders (thrombocytopenic purpura, Henoch-Schonlein purpura, acute polyneuropathy, Sjorgen syndrome, autoimmune thyroiditis or food allergies). Also, in the case of infection by cytotoxic strains it may cause Raynaud's syndrome, idiopathic migraine or cerebrovascular atherosclerosis.

Helicobacter pylori is a pathogenic bacteria that has had a long relationship with the human species, as it colonized the

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gastric mucosa even tens of thousands of years ago, due to its exceptional adaptation in this ecological niche. The acidic gastric environment of the human host is a natural barrier against the digestive pathogens, and those germs, viruses or parasites that have the necessary strength to survive the stomach hydrochloric acid solution are merely transiting this section of the digestive tract to colonize other sites, much less hostile to their survival. In contrast, *H. pylori* remains and persists indefinitely in the gastric mucosa in the absence of treatment, this being the environment where it exists and where it multiplies, a competition-free environment for a bacteria that has adapted to the gastric protective factors: a variable acidic pH, fluctuations in the concentration of nutrients, mucus secretion, the host's immune response, chronic inflammation, oxidative stress and the presence of toxic metals.

The ability to grow in this special acidic environment results from a combination of outstanding physiological properties of the bacteria, which facilitate increased motility, chemotaxis and infiltration in the mucus, attachment to the stomach's epithelial cells, the avoidance of the host's immune system and long term proliferation.

The relationship between the *H. pylori* bacteria and the human host can be explained in three stages, each involving specific factors.

In the first stage *H. pylori* crosses the stomach and enters in the gastric mucosa due to the bacteria's mobility, microaerophilism, urease and ammonia. The next step of joining the gastric epithelium and colonization of the mucosa leads to long-term resistance and chronic infection due to factors such as lipopolysaccharide O, bacterial adhesins,

Hop proteins, Vac A cytotoxin, gastric acid secretion inhibiting factors(AIF-1, AIF-3), with the contribution of urease and ammonia. This step is facilitated by the genotypic and phenotypic diversity of *H. pylori*.

In the third stage we can observe the harmful action on the gastric mucosa by means of Vac A cytotoxin, the cytotoxin-associated protein (Cag A), cag-PAI pathogenicity island, the inhibition of hydrochloric acid secretion and stimulation of the expression of proinflammatory cytokines (IL-1, IL-6, IL-8, interferon- γ , TNF- α).

2. Colonization of the stomach

In general, bacterial colonization of the stomach is prevented by means of the peristalsis, gastric mucus and gastric acidity. Specific *H. pylori* can fight against these protective mechanisms through their own resistance mechanisms such as the excellent mobility provided by a spiral form and a *unipolar* bundle of two to six sheathed *flagella*, microaerophilism, and the presence of the bacterial enzyme urease.

2.1. Mobility

The mobility of *H. pylori* is affected by the molecular weight of the glycoproteins that make up the gastric mucus [24]. A larger molecular weight means a greater viscosity and thus lowering the bacteria's mobility. Bacteria adapted even to this difficult situation, the changes that occurred prevailing upon the increased viscosity. The spiral form is amplified during travel and flagella are those that propel bacteria effectively through the gastric mucus according to the

concentration gradients of various substances.

Urea, sodium bicarbonate, and some amino acids are chemotactic attraction factors for *H. pylori*. This way an entire signaling system which is connected to the flagella engine is activated, by means of the MCP (methyl-accepting chemotaxis) protein. Each flagellum is composed of two different flagelline types: a major Fla-A subunit the size of 53 kiloDaltons and a minor Fla-B subunit of 54 kiloDalton [10]. The two flagellines are wrapped in a protein membrane. The synthesis of Fla subunits A and B is encoded by the homonymous genes. It has been shown that all molecular variants of the Fla A gene are responsible for the emergence and development of lesions in the gastric mucosa, since all these gene variants induce synthesis of pro-inflammatory cytokine IL-8 in various amounts [28], [38].

Full operation of flagella is essential and indispensable in the colonization of the gastric mucosa [43]. *H. pylori* strains which lack flagella or have malfunctioning flagella can't colonize the stomach or have great difficulty in permanently settling in the host organism.

2.2. Microaerophilism

H. pylori is classified as a microaerophilic germ, therefore vulnerable to the oxidative stress and to reactive oxygen species [21]. The bacteria's development can be achieved in the complete absence of oxygen. The environmental oxygen concentration at which they proliferate in ideal conditions is between 5% and 10%. This is an environment in which it adapted by means of an elaborate antioxidant system. In

vitro, growth was also favored in environments with hypercapnia [8].

2.3. Urease

As a result of the chemical reaction of hydrolysis of urea by urease, ammonia and bicarbonate occur, acting as buffers. Thus, around the bacterial cell the environment becomes alkaline [7]. The resulting pH gradient around the bacteria amplifies bacterial motility by 40% [23] and facilitates the crossing of the gastric mucus, the gastric epithelial cell layer being reached easier. Here the pH is close to neutral and provides the optimum conditions for bacterial proliferation.

Urease is a metalloenzyme present both in the cytoplasmic membrane surface and in the cytoplasm, predominantly in the cytoplasm in the case of young bacteria, and close to the membrane surface for mature bacteria [49]. In the cytoplasm, the ammonia resulting from urea hydrolysis buffers the hydrogen ions penetrating from the outside.

Urease has a molecular weight of 600 kiloDaltons, an enzyme of this size being a strong antigen. Its hexamer structure is composed of structural subunits Ure A and Ure B [26]. The encoding is done by many genes: Ure-A and Ure-B encode homonymous subunits; structural genes Ure-E, Ure-F, Ure-G and Ure-H encode the synthesis of proteins necessary for coupling nickel; Ure-I gene has a dual role: encoding the synthesis of the membrane protein that constitutes the membrane channel for the urea and acts as a detector of low pH - in an acidic environment the urea channel opens, urea enters into the cytoplasm, and the chemical reaction of urease hydrolysis takes place [48]. The resulting ammonium ions have a buffering

effect on the hydrogen ions that enter the cytoplasm [41].

The enzymatic action of urease is only possible in the context given by the presence of nickel ions [9]. Nickel is a cofactor of metalloenzymes required for hydrogenase as well. Nickel is present in very low quantities in the human body, with a serum level between 2 and 11 nM, and ingestion varies significantly with foods of different types and origins [9], [44]. Therefore, *H. pylori* requires an effective mechanism to supply nickel, manifested by the presence of several protein carriers such as NixA and several others with an unclear role as of yet [32]. The latter include the abcCD system [19], DPP dipeptide permease, and one or two histidine-rich proteins (HPN - histidine - rich proteins) near transport proteins with high affinity for nickel [17], [33]. NixA protein is localized in the plasma membrane and has a definite role in nickel transportation for which it has a high affinity [14], [16], [43], [47].

3. Adherence to the gastric epithelium and permanent colonization of the mucosa

Studies on mice have shown that *Helicobacter pylori* exhibits a preference for colonization of the gastric mucosa where it has no parietal cells [45]. The human body is colonized preferentially by antrum pylorus, since at this level the parietal cells are not present and, in addition, colonization is favored by the slightly alkaline pH.

Although bacteria are mobile in the gastric mucus, a part of them (20%) binds to gastric epithelial cells [26] by means of bacterial adhesins. There is a variety of adhesion molecules specific to each type of

bacterial strain, as well as a variety of host receptors and the interaction between them varies depending on the combination of the bacterial strain and the configuration of the host receptors. However, the presence of lectin-like adhesins on the surface membrane is characteristic of all strains of *H. pylori*, which is essential for adhesion, along with membrane proteins Hop, vacuolated cytotoxin Vac A and lipopolysaccharide O.

The target receptor on the epithelial cell surface where the attachment of the bacteria takes place is a Lewis glycosphingolipid [30]. In the case of *H. pylori*, the presence of Lewis antigens determines an immune response materialized by the appearance of anti-Lewis type antibodies. The gastric epithelial cells express protein receptors that resemble anti-Lewis antigens, and anti-Lewis antibodies can interact with the epithelial cells of the host, this phenomenon varying according to the host's phenotype. In the mucosal areas where the adhesion of *H. pylori* takes place, the secretion of IL-8 cytokine is also stimulated [42]. After joining the epithelial cell, *H. pylori* can insert into it and thus resist antibiotic treatment, returning later to the surface and proliferating [1].

3.1 Lipopolysaccharides

Gram-negative bacteria contain lipopolysaccharides in their cell wall which are in fact phosphorylated glycolipids involved in cellular integrity. At the same time, lipopolysaccharides represent antigens for the immune system of the host. The host's antibodies bind to these antigens, act in pyrogenesis and have a toxic action locally. Their release occurs

in two situations, namely during bacterial decay and replication.

By means of lipopolysaccharides, *H. pylori* has a high affinity for the basal lamina which becomes exposed in areas where epithelial cells were destroyed. The interaction between *H. pylori* and the basal lamina ultimately leads to lesions [27], [35].

The mechanisms of lipopolysaccharide toxicity are multiple. One of them consists in the coupling of somatostatine receptors, blocking them, both phenomena leading to increased secretion of gastrin. Another mechanism is the stimulation of the release of Interleukin-8. The secretion of pepsinogen and increased production of selectins are also stimulated. The presence of lipopolysaccharides also interacts with DNA synthesis in ECL cells (enterochromaphine like), leading to their proliferation in excess.

The molecular weight of lipopolysaccharide varies among different strains of *H. pylori*. There are smooth lipopolysaccharides forms which have high molecular weight and rough forms, with low molecular weight [40]. In the case of strains isolated in the stomach, most of the lipopolysaccharides had high molecular weight, while in the strains grown in the laboratory mainly low molecular lipopolysaccharide [34] are present.

Structurally, lipopolysaccharide consists of the following several elements: a polymer chain (O), a central region ("core") and stable lipid A. The O polymer chain exhibits a strong antigenicity. On the one hand it mimics the Lewis antigen, which results in concealment of bacteria from the immune system of the host. On the other hand, because of mimicry, some antibodies cross-react with protein components of the human host, leading to

Guillain-Barre syndrome [3], [50] and the development of autoimmune diseases related to infection by *H. pylori* [50].

The central region, "core" consists of 10 to 15 oligosaccharides [12] by means of which the activity of stable lipid A is modulated. Stable lipid A mediates the bacteria's interaction with the environment [36]. Through phosphorylation and fatty acid substitution, spatial changes occur that decrease the antigenic potential and promote long-term survival of the bacteria [37]. It has been observed that *H. pylori*'s lipopolysaccharides have a beneficial role in the sense that they provide some protection against lesions caused by alcohol [25].

3.2 Pathogenicity Island Cag A

Cag-PAI pathogenicity island is a particular nucleotide sequence in the bacterial DNA that contains 31 genes, including the gene encoding the Cag A protein [6]. The island is present in the virulent strains of *H. pylori* [5] and it can have variations in three regions.

There are variations of Cag A positive strains by geographical area, some variants are more virulent and are associated with a higher prevalence of gastric cancer in studied populations [5]. The gene represented by the above-mentioned nucleotides encodes a protein with immunogenic properties – Cag A protein – with a high molecular weight of 145 kDa, against which the host organism secretes anti Cag A antibodies (so there is the possibility to verify the presence of *H. pylori* by blood tests).

Anti Cag A antibodies have cross-affinity for protein components of the vascular endothelium and smooth muscle fibers of blood vessel walls, which is

correlated with the occurrence of atherosclerosis in patients infected with Cag A positive *H. pylori*. [15]

Cag A protein plays a role in bacterial adhesion to the epithelium of the stomach, and may be transferred to gastric epithelial cells, granulocytes and macrophages [2]. In the epithelial cells it undergoes phosphorylation and it stimulates the synthesis of pro-inflammatory cytokines. Severe lesions can result from these phenomena, and the presence of a strong inflammation of the stomach leads us to the conclusion that strains exhibiting Cag A pathogenicity island have an increased potential to induce duodenal ulcers and gastric cancer.

The cag A protein also stimulates cell proliferation by coupling a growth factor receptor [31], which is the main mediator for this protein. The virulence of Cag A positive *H. pylori* strains is higher in the cases where Vac A vacuolated cytotoxin is associated. Based on these two virulence factors, *H. pylori* strains are classified into two main types:

- type I *H. pylori*, located in the extreme virulence spectrum, Cag A and Vac A positive. Often this type of *H. pylori* is associated with ulcers and gastric cancer.
- type II *H. pylori* with low pathogenicity, which lacks both Cag A and Vac A.

Between the two extremes, the following were classified as intermediate pathogenic strains: type III *H. pylori* - *Cag A positive and Vac A negative*, and type IV *H. pylori* - *Cag A negative and Vac A positive*.

3.3 Vacuolated cytotoxin - Vac A

Vacuolated cytotoxin or Vac A is another major pathogenicity factor expressed by *H. pylori*, present in 60% of the strains, although the gene which

encodes it, also named Vac A, is found in all *H. pylori* strains [4]. Its secretion is modulated by environmental pH, switching on when the pH falls below 6. Simultaneously, the production and secretion of urease also starts [11].

Structurally, it is a protein with a molecular weight of 95 kDa, which, once having reached the stomach epithelial cells it inserts into the membrane, where acts as a selective anionic channel. This channel permits the export of bicarbonate ions and urea from the epithelial cells, helping *H. pylori* to better survive in the stomach [46]. The term "vacuolated" cytotoxin is given due to the ability to induce the formation of vacuoles in the epithelial cells of the stomach. In experimental studies on rats it was also observed that Vac A protein causes vasoconstriction to the stomach lining capillaries, increases vascular permeability and increases platelet and leukocyte aggregation [22].

H. pylori strains expressing the Vac A protein induce apoptosis by modulating the signals by which the C cytochrome is transferred from the mitochondria into the cytoplasm [29]. It also relaxes the intercellular tight junctions of the epithelial cells, allowing nutrients to pass into the stomach [39], towards *H. pylori*, and prevents the host's immune response, resulting in long-term colonization of the stomach.

Other changes to the gastric mucosa that act as favoring factors for long-term *H. pylori* colonization include antioxidant enzymes such as catalase and superoxide dismutase, collagenase, mucinase, protease, phospholipase A2, phospholipase C, sphingomyelinase and proinflammatory mediators such as interleukin 1, interleukin 6, interleukin 8, interferon-gamma and tumor necrosis factor alpha [13], [18].

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