

EDITORIAL

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Helicobacters: from Bacterial Adaptation to Pathogenicity

Helicobacters: od adaptacji do patogenności

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Abstract

Bacteria from the *Helicobacter* genus represent a good example of adaptation to a specific ecological niche. They have the characteristics of bacteria living in the mucus of the digestive tract (microaerophily, spiral shape, motility). Some of them probably co-evolved with the stomach when this organ appeared in vertebrates: they are the gastric *Helicobacters* with the presence of an abundant urease and a pore channel typical for this group. The human *Helicobacter*, *Helicobacter pylori* in contrast to the others induces gastric inflammation which paves the way for severe diseases, e.g. ulcers and cancers. Its specific property is partially explained by acquisition of a *cag* pathogenicity island and production of a cytotoxin. More information of how a commensal may become a pathogen may emerge from studies of this group of bacteria (Adv Clin Exp Med 2005, 14, 1, 5–10).

Helicobacters are members of the Epsilon group of Proteobacteria. They are closely related to the family *Campylobacteriaceae* [1]. These bacteria emerged recently as an important group of bacteria largely neglected in the past, which has specific characteristics attesting to its adaptation to a particular ecological niche, i.e. the digestive tract of animals and men.

In addition to these properties of adaptation to life in the digestive mucosa, some *Helicobacter* species developed characteristics allowing them to live in the stomach, especially urease. *Helicobacter pylori* (*H. pylori*), the human adapted *Helicobacter*, is the only gastric *Helicobacter* which induces inflammation. This is mainly due to the acquisition of a pathogenicity island, namely *cag*, which is found in some of the strains as well as to the production of large amounts of a cytotoxin.

In this article the author will review the characteristics of the strains linked to adaptation and pathogenesis with an emphasis on the particularities of *H. pylori*, the species by far the most studied.

Adaptation of Helicobacters to Their Ecological Niche

Helicobacters in the Digestive Tract

Helicobacter ancestors were probably bacteria living in the gut of vertebrates. They developed characteristics allowing them to live in the mucus layer close to the mucosal cells: microaerophily, spiral shape, flagella and possibly adhesins [2].

Microaerophily

To be able to survive in the digestive mucosa where there is low oxygen pressure, it was necessary for the bacteria to adapt by developing a specific enzyme equipment. Most *Helicobacters* need a relatively narrow range of oxygen, and the 21% oxygen present in air is toxic for them [3]. The numerous *H. pylori* cells usually present near the intercellular spaces may correspond to the best environment for them.

Morphology

Interestingly, the wavelength of the Helicobacter body spiral is dependent on the viscosity of the environment. When the bacteria are in water they look like bacilli, and when viscosity increases like in mucus the spiral shape appears and increases. It allows them to improve their motility.

The motility and capacity to colonize the stomach is mainly due to the flagella present at the extremity (up to 6). The flagella structure and genetic determinant have been largely studied for *H. pylori* and to a lesser extent for *H. mustelae*. Indeed, the flagella are covered with a phospholipid layer which protects them from gastric acidity. Otherwise, the flagellin would be depolymerized.

H. pylori flagella comprise 2 subunits: FlaA and FlaB [4], and the 2 corresponding genes are mandatory to motility, as proved by inactivation of these genes [5]. However, many more genes are involved in the regulation production and make up of the flagella structure, attesting to the fact that these elements contribute to a highly adapted function of the bacteria. A regulation by a mechanism of an on/off switch due to a repeated sequence has been described [6].

The flagella apparatus is linked to chemotaxis because these bacteria can be attracted by specific substances such as urea.

Adhesins

Adhesins are an important factor for colonization although they are not absolutely necessary; for example, gastric Helicobacters other than *H. pylori* do not adhere to the gastric cells. Therefore this element is clearly on the borderline between the colonization function and the pathogenicity factor, because a close association between the bacteria and the cells allows a more efficient release of the pathogenicity factors.

By *in silico* analysis of the *H. pylori* genome several groups of genes potentially involved in adherence have been discovered, including 19 lipoproteins and 32 outer membrane proteins (omp) or Helicobacter outer proteins (Hop). Among the lipoproteins, AlpA and AlpB (adhesion associated lipoproteins) have been described but their cellular receptor remains unknown [7]. Among the proteins, the most thoroughly studied is Bab (blood group antigen-binding protein). By using a transgenic mouse model it was shown that the Lewis B antigen expressed on the gastric cells was indeed its cellular receptor and that adherence allowed chronic atrophic gastritis to develop. The *babA2* gene which encodes the BabA protein has a G + C% different from the *H. pylori* genome and therefore

appears to have been acquired from other bacteria by horizontal transfer [8].

Most genes encoding potential adhesins in *H. pylori* exhibit a phase variation. One example is *hopZ* whose regulation is due to a CT repeat in the 5' coding region. The HopZ receptor is also unknown [9].

Another example is *sabA* encoding for SabA (sialic acid binding adhesin A) whose expression is regulated by a CT repeat at the coding region site [10] and whose receptor appears to be a sialylated Lewis X.

Enzymes

In order to survive the inflammatory process which may occur in the digestive tract, Helicobacters have developed mechanisms allowing them to escape the compounds generated by phagocytes, especially the enzymes: catalase and superoxide dismutase. The catalase is expressed both inside and outside of the cell and in the periplasmic space [11]. The superoxide dismutase is localized at the bacterial surface. Mutants of the superoxide dismutase have been shown to be unable to colonize in the mouse model [12].

However, the presence of these enzymes correlates with gastric epithelial cell DNA alteration indicating that they can also be considered as virulence factors [13].

Lipopolysaccharide (LPS)

In order to avoid the host immune response and to colonize the gastric mucosa, *H. pylori* has acquired host molecules such as Lewis antigens. The molecular mimicry is indeed very special to *H. pylori* but has also been described for *H. mustelae* [14]. Lewis X and Y antigens similar to those expressed on erythrocytes are present in 80% of the strains. One consequence is the low toxicity of *H. pylori* LPS in comparison to LPS from other bacteria such as *Enterobacteriaceae*. However, the Lewis antigen expression is not stable in a given strain because the different genes involved are submitted to phase variation [15].

The consequence, which has been suspected but not proven, is indeed autoimmunity with the risk of atrophy [16] and internalization of the bacteria in gastric cells [17].

Helicobacters in the Stomach

The stomach evolved in vertebrates from the anterior gut to a specialized organ devoted to protect the body from the introduction of dangerous

bacteria from the environment. This could be achieved by the production of acid and occurred probably 350 million years ago. It is most likely that the evolution of gastric Helicobacters paralleled this anatomic and physiologic evolution which would explain why most animal species now seem to possess their own *Helicobacter* species. For example, two new *Helicobacter* species have recently been found in harp seals [18]. The main Helicobacter adaptation to this environment is the acquisition of a urease. Urease may exist in enteric Helicobacters but in this context its activity is similar to other bacterial ureases. The peculiarity of gastric Helicobacters is the activity and abundant production of their urease (up to 6% of the total protein produced) allowing the use of a low amount of urea diffusing from the gastric mucosa in order to produce ammonia and buffer the acidic pH [19]. *H. pylori* urease is formed by 2 proteins, UreA and UreB, linked to nickel ions. In addition to the *ureAB* operons, there are other genes essential for nickel ion incorporation [20], including the important *ureI* gene encoding a pore gate protein activated at acidic pH which allows the urease to diffuse when the bacteria is in an acidic environment [21]. Acquiring this *ureI* gene may be the key factor in the evolution process from an enterohepatic to a gastric Helicobacter, as previously proposed [22]. However, gastric Helicobacters being the only bacteria able to survive in such an environment they have also lost their adaptation to vital competition with other microorganisms and consequently cannot survive in the intestine.

Pathogenicity of *Helicobacter pylori*

While most if not all of the Helicobacters do not induce inflammation in their host, in humans *H. pylori* always induces an important infiltrate of inflammatory cells composed of lymphocytes and polymorphs. This is the chronic active gastritis which is almost pathognomonic of the presence of this bacterium in the stomach.

Some of the factors described as colonization factors may play a role in this respect to induce a low inflammatory response. This basic inflammatory response is always present but for ethical reasons (the need to perform endoscopy) has not been extensively studied in subjects without any symptoms. Since the discovery of the importance of toll-like receptors (TLRs) in determining the innate response to bacteria, some studies have been performed on *H. pylori* with controversial results. Indeed, it appears that TLR5 which senses flagellin does not recognize *H. pylori* flagellin [23] and TLR4 does not recognize

H. pylori LPS [24]. However, more studies need to be carried out on this topic and, the polymorphism of TLRs must also be taken into account.

Much data has been gathered from patients with digestive symptoms and it is interesting to note that the majority of the strains do possess a pathogenicity island (PAI) namely *cagPAI*, a subgroup of which produces a cytotoxin (VacA).

cagPAI

The *cagPAI* corresponds to a region of the chromosome, approximately 40 Kb, encoding 30 open reading frames (ORF) including homologs of genes involved in the formation of a type IV secretory apparatus in *Agrobacter tumefaciens*, i.e. a multiprotein complex which allows the injection of a protein, e.g. CagA in the gastric epithelial cell [25–27]. This injection leads to a cascade of cellular events most of which have been nicely described. Briefly, on one side the translocated CagA is phosphorylated by cellular kinases which leads to cytoskeleton reorganization. The cells take on a different shape (“hummingbird” phenotype) and a cellular proliferation may occur. On the other side, there is an activation of the signaling molecule nuclear factor κ B which leads to the production of proinflammatory cytokines such as interleukin 8 [28]. In terms of evolution, this *cagPAI* was most likely acquired from exogenous bacteria. It has a G + C% different from the rest of the genome (35% vs. 39%, respectively) and it is inserted in the glutamate racemase gene.

Now that *H. pylori* genomes have been more thoroughly studied, it appears that this *cagPAI* is present in most strains found in the Far East while only in some of those found in the rest of the world but the selection pressure leading to this finding is not known.

It is also noteworthy that the *cagPAI* is submitted to rearrangements, insertions and deletions as is the rest of the genome. In some strains the *cagPAI* has been separated into 2 parts by an insertion sequence (IS 605). In other strains part of the genes have been deleted. Despite the fact that *cagA* is a gene of major importance in the *cagPAI*, again with a role of repeated sequences [29], it is not possible to rely solely on its detection by PCR to conclude that it is a functional *cagPAI*, because of the complexity of its action.

Other Proinflammatory Proteins

An IL-8 production can be shown in some *cagPAI* negative *H. pylori* strains. This is indeed due to an outer membrane protein namely OipA

[30]. Different experiments including the production of OipA negative mutants have shown that OipA was not an adhesin but had a role in gastric inflammation.

Another protein, IceA, induced by contact with the epithelium has also been studied. The presence of the *iceA1* allele is associated with ulcer evolution [31].

Finally a family of proteins, Hop, has been studied. As previously described, some of them are adhesins and others are porins, but it seems that certain ones like HopZ and HopQ also have a pathogenic potential [32].

Vacuolating Cytotoxin VacA

The gene coding for the cytotoxin VacA is only found in *H. pylori* and is present in all *H. pylori* strains. However, the cytotoxin is produced in less than 50% of the strains. This is due to the polymorphism of the gene, and especially to the signal peptide region(s). When s1 is present, VacA is produced; the contrary is true with s2 [33].

In terms of evolution, it is interesting to note that there is an association between the s1 subtype and geographic distribution: s1c is only found in Asian strains, s1b is the main subtype found in the Iberian peninsula and Latin America, while s1a and s1b are found in Europe and North America, evoking a strain adaptation to the infected host [34].

VacA is definitely a pathogenic factor which appears to have a proapoptotic effect by acting at the mitochondrial level [35]. In addition, recent data suggest that it could down regulate the Th1 immune response [36].

It has been found associated with the presence of the *cagPAI*, reflecting a positive selection of the strains harboring both pathogenicity factors during evolution.

In summary, *Helicobacter* species have special characteristics testifying to their adaptation to a special ecological niche, the digestive mucosa of mammals. Such living conditions can hardly be reproduced *in vitro* which makes it difficult to grow and to study many of the *Helicobacter* species. A further species adaptation occurred when the stomach appeared with its unique acid environment. Gastric *Helicobacters* are among the few bacteria to survive there, thanks to their abundant urease. It is a striking feature that pathogenicity has occurred only regarding the human *Helicobacter*, i.e. *H. pylori*. This is the only species which constantly induces an inflammation, leading to severe diseases such as ulcers and cancers. The acquisition of the *cagPAI* is undoubtedly an important factor but does not explain the whole story. There is no doubt that comparative genomics will soon reveal important mysteries of this fascinating group of bacteria.

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