Epidemiology of *Helicobacter pylori* and Mechanisms of Carcinogenesis

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1 Epidemiology

Soon after the discovery of *H. pylori*, numerous studies based on serological surveys highlighted an increasing prevalence of *H. pylori* infection with age within a population, which was inversely related to the socio-economic status [14]. This was related to the so-called cohort phenomenon: each age group has a different prevalence of an infection acquired primarily during childhood and lasting lifelong. The risk of acquiring the infection has decreased over the years due to improvement in socio-economic conditions.

Regarding Europe, the prevalence of *H. pylori* infection according to the year of birth has decreased quickly and dramatically in countries from Northern Europe (e.g. Finland), whereas it remains high in some countries from Southern Europe (especially Portugal) [11]. Indeed, *H. pylori* infection remains a frequent and early event in Portugal [13].

A gastric biopsy-based study conducted in the Brussels area (Belgium) over a 20-year period has also nicely shown that the prevalence was much higher in children (0–9 years old) with a North African origin compared to children with Western European origin. Therefore, these data show the effects of time, age and ethnicity on

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the prevalence of *H. pylori* infection and its complex heterogeneity in the same cosmopolitan urban area [15].

It has been estimated that approximately 50 % of the world's population harbours *H. pylori*. Are there any populations which are *H. pylori* free? The answer is clearly no, even if some countries show surprisingly low prevalence such as the Malay population [23].

The main reservoir remains the human stomach. The transmission between humans is mainly intrafamilial. The role of parents has been nicely investigated in particular by Dominici et al. in a prevalence-based study which assessed the presence of IgG antibodies; results showed a higher prevalence in children living in families where the two parents were infected compared to those living in a one parent-infected family. The predominant role of the mother in transmission was also noticed [5]. The study of transmission in families was further investigated on three families using microarrays and sequencing of two housekeeping genes [22]. The authors showed that two to five strains circulated within a given family. Identical strains were present in at least two members of all three families supporting the accepted model of intrafamilial transmission, but they also showed that sibling-to-sibling transmission and acquisition of *H. pylori* from outside the family were also probable.

The possible vehicles of *H. pylori* transmission are vomit, saliva and faeces; therefore, the routes of transmission are mainly gastro-oral, oral-oral or faecal-oral. *H. pylori* can indeed be found in viable form in vomit [21], and therefore, we now have strong arguments in favour of a gastro-oral transmission.

The publication of anecdotal reports of positive culture from saliva as well as PCR results points to saliva as another source of *H. pylori* transmission following regurgitation and vomiting.

H. pylori can be detected in faeces of infected individuals, and accordingly, an antigen stool test or even PCR detection can be performed to diagnose the infection. However, is *H. pylori* viable in faeces? Although *H. pylori* stool culture is extremely difficult, it is possible in case of a short intestinal transit that can be induced or spontaneous [21]. Therefore a faecal-oral transmission is possible mainly in developing countries where frequent diarrhoeal episodes are found, faecal hygiene is lacking and water to be used in the household is not well treated.

2 *H. pylori* Infection at the Origin of Malignant Diseases

H. pylori is a non-invasive bacterium that survives and multiplies in the mucus and on the gastric epithelial surface thanks to the production of a urease which allows it to neutralise the mucus acidity in the microenvironment.

H. pylori infection consistently induces a chronic inflammation of the gastric mucosa. In approximately 1 % of cases, the infection leads to two types of stomach cancer, gastric adenocarcinoma and gastric MALT lymphoma (Fig. 1).

At the cellular level, *H. pylori* infection leads to a Th1 immune response and to a chronic gastritis, followed by an increase in the apoptosis of the gastric epithelial cells resulting in atrophy and leading to a compensative cellular hyperproliferation and an alteration of the differentiation, which is at the origin of the intestinal

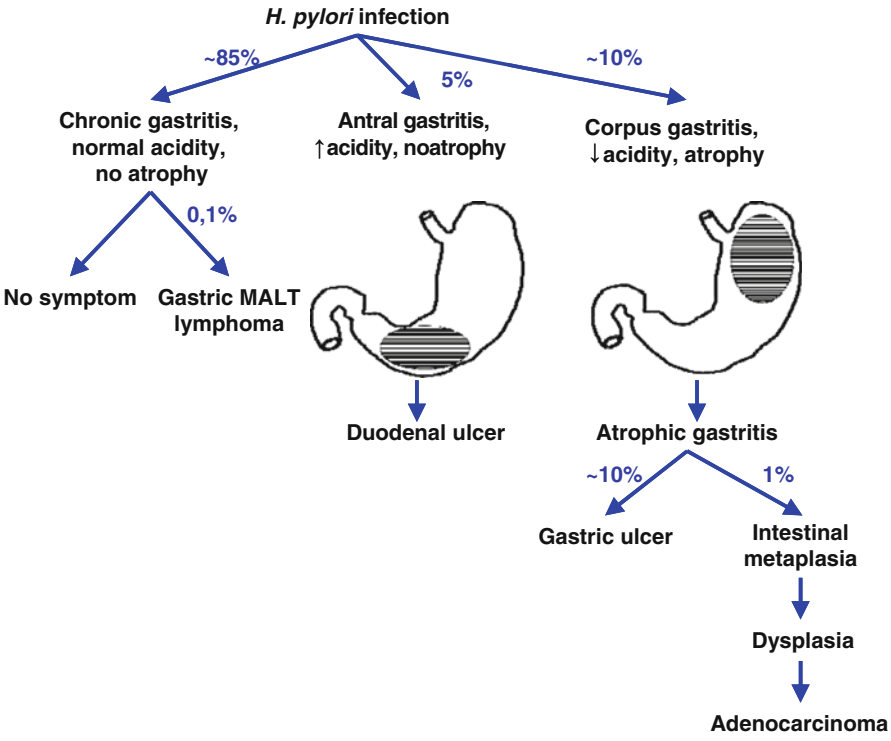

Fig. 1 Gastric pathologies induced by *Helicobacter pylori* infection

metaplasia [3]. These metaplastic lesions can further evolve into dysplasia, in situ carcinoma and finally an invasive adenocarcinoma.

Gastric MALT lymphoma (GML) caused by *H. pylori* infection in humans is one of the most beautiful examples of the role of a chronic infection and inflammation in cancer [26]. MALT-type lymphomas are the most frequent extranodal entities usually found in organs normally devoid of lymphoid tissue such as the stomach. The development of GML in humans is directly related to infection by *H. pylori* and appears in nearly 1 % of infected individuals. The association of *H. pylori* infection with GML is strong and causal: it is indeed currently the only cancer which can be cured at an early stage by an antibiotic treatment [25]. Chronic antigenic stimulation exerted by *H. pylori* on the gastric mucosa induces dense lymphoid infiltrates containing small centrocytic B cells expressing several surface markers typical of marginal zone B cells in normal MALT.

No bacterial virulence factor has yet been clearly associated with this disease [12]. A better understanding of the pathophysiology of this lymphoma has been hampered by the difficulty in obtaining primary xenografts from surgical specimens of GML patients. Based on the use of animal models, it is now admitted that the inflammatory, molecular and immunological contexts of the host prior to *H. pylori* infection are probably essential contributors to GML pathogenesis [4].

3 *H. pylori* Virulence Factors

H. pylori has developed a unique set of factors (Table 1), actively supporting its successful survival and persistence in its natural hostile ecological niche, the human stomach, throughout the individual's life, unless treated. In the human stomach, the vast majority of *H. pylori* cells are moving in the mucus layer lining, but a small percentage adhere to the epithelial cell surfaces.

H. pylori is one of the most genetically diverse bacterial species known, and it is equipped with an extraordinarily large set of outer membrane proteins (OMPs), whose role in the infection and persistence process is crucial. The large group of OMPs contains adhesins (BabA and SabA), adherence-associated lipoproteins (AlpA/B) and inflammatory OMPs (OipA or HomB) that mediate *H. pylori* binding to the host cell membrane. Some of these OMPs are associated with more severe pathologies, such as HomB in ulcers [19] and cancer [10]. The *babA* genotype correlates with the highest incidence of ulceration and gastric cancer [24].

One of the most extensively studied toxins produced by *H. pylori* is the vacuolating cytotoxin A (VacA). Infection with *H. pylori* strains containing the toxigenic allelic s1 form of VacA is associated with an increased risk of peptic ulceration and gastric cancer. Once internalised into the cell, VacA induces severe "vacuolation" characterised by the accumulation of large vesicles. VacA also exerts effects on target cells, including disruption of mitochondrial functions, stimulation of apoptosis and blockade of T-cell proliferation [20].

The oncoprotein CagA encoded by the *cag*A gene of the *cag* pathogenicity island (*cag*PAI) is one of the most studied virulence factors for *H. pylori*. The *cag*PAI encodes proteins assembling a type 4 secretion system (T4SS), which interacts with

Table 1 List of bacterial factors of *H. pylori* associated with gastric adenocarcinoma

Name	Function	Targets	Bacterial genotype associated with cancer
BabA	Adhesin	Lewis antigen	*bab*A2+
SabA	Adhesin	Sialyl-Lewis antigen	*sab*A+
OipA	Adhesin	Unknown	*oip*A+
HopQ	Adhesin	Unknown	*hop*Q1
HomB	Adhesin	Unknown	*hom*b+
VacA	Vacuolating cytotoxin	RTPβ receptor	*vacA* s1m1
T4SS	Type 4 secretion system encoded by the *cag*PAI	β1 integrin	*cag*PAI+
CagA	Oncoprotein	Cell-cell junctions proteins (ZO-1, JAM and E-cadherin), β-catenin, kinase PAR1, Src kinases, Erk1/2 kinases, SHP2 phosphatase, c-Met receptors	*cag*A+

cagPAI cag pathogenicity island

the α5ß1 integrin at the surface of the gastric epithelial cells and allows the injection of different bacterial effectors including CagA and pro-inflammatory components of the bacterial peptidoglycan directly into the cytoplasm of the host cell [29]. CagA thereby affects several signalling pathways and cell proliferation and differentiation states [1]. A direct causal link between CagA and gastric carcinogenesis was proven in transgenic mice expressing CagA: these mice spontaneously develop gastric polyps and adenocarcinoma, revealing CagA as the first bacterial oncoprotein [18].

4 *H. pylori*-Mediated Inflammatory Response

The role of inflammation in *H. pylori* infection is also of major importance as it triggers the outcome of this chronic infection.

The activity of the inflammatory response (polymorphonuclear and lymphoplasmacytic infiltration) as well as the preneoplastic lesions such as atrophy or intestinal metaplasia is evaluated during histological examination of gastric biopsies according to the Sydney system. The development of chronic atrophic gastritis and other preneoplastic lesions is associated with decreased pepsinogen I/II ratio, increased plasma gastrin concentration and a Th1 polarisation of the immune response (IFN, IL-1β, IL-8 and TNF) that will inhibit somatostatin production and therefore stimulate gastrin production. The TNF and IL-1β also inhibit the acid production by the parietal cells.

The tendency of an immune response to become polarised towards Th1 or Th2 effectors is influenced by a combination of host genetic factors and the type and amount of antigen that is encountered. IL-12 and IFN-γ are important for induction of Th1 response, whereas IL-4 and IL-13 play critical roles in promoting Th2 response. However, in many cases, it is still not understood why a Th1 or Th2 response is preferentially induced. Of particular interest, the immune response to Helicobacter infection can be modulated experimentally in mice by co-infection with nematodes towards a Th2 type that protects against gastric atrophy [8]. Such modulation is mediated by downregulation of the Th1 cytokines TNFα and IL-1β and higher levels of the Th2 cytokines IL-4 and IL-10 [9].

Polarisation towards a Th1 profile can contribute to the development of peptic ulcers and other severe mucosal pathology, while on the other hand, activation of a Th2 cell response results in amelioration of the gastritis. Therefore, it has been deduced that an uncontrolled Th1 cell response to *H. pylori* infection results in persistence of inflammation and disease, whereas, in contrast, a Th2-mediated response reduces the pro-inflammatory immune effects [17].

In humans, the innate immune response caused by the infection is not sufficient to eliminate the pathogen. Regulatory T cells recruited in the gastric mucosa during infection could also play a role in the development and persistence of chronic inflammation via the production of cytokines that regulate the inflammatory response such as TGFβ and IL-10. Moreover, immune response contributes to chronic gastritis ultimately leading to the development of more severe disease in some individuals.

H. pylori strains from East Asia are associated with a high cancer risk and African strains with a low cancer risk in African populations despite the fact they are highly infected. This so-called African enigma probably reflects the modulation of the inflammatory process initiated by the infection, towards a non-neoplastic outcome. The available scientific evidence supports the role of the following factors, listed in order of their possible importance in explaining the enigma: the oncogenic potential of different bacterial genotypes, the modulation of the immune response to Helicobacter infection towards a Th2 type and dietary influences and host genetic susceptibility [9].

5 Mechanisms of *H. pylori*-Mediated Gastric Carcinogenesis: The Disease

Gastric adenocarcinoma is a poor prognosis disease, with a survival rate of less than 20 % at 5 years, and apart from surgery (partial or total gastrectomy), no specific therapy is recommended. Gastric adenocarcinomas are histologically heterogeneous. Except for rare cases of family cancers found in young adults associated with mutations in the *cdh*1 gene, gastric adenocarcinomas are sporadic, occur in people over 60 years old and almost always develop over a mucosal inflammation resulting from chronic infection by *H. pylori*.

Preneoplastic lesions and gastric cancers are induced by *H. pylori* infection. The stomach cancers are essentially adenocarcinomas originating from the transformation of gastric epithelial cells and in rare cases (approximately 5 %) sarcoma (gastrointestinal stromal tumours or GIST) of stromal origin. Distal gastric adenocarcinomas associated with *H. pylori* infection can be separated into two main histological types: the diffuse type (70 %) and the intestinal type (30 %). The diffuse type is a poorly differentiated adenocarcinoma wherein glandular architecture disappears. Diffuse type adenocarcinomas are in most cases associated with sporadic or inherited mutations in the *cdh*1 gene encoding E-cadherin, which inhibit the expression of this molecule at the adherent cell junctions, producing often highly invasive independent cell tumours. Adenocarcinomas of intestinal type have a preserved glandular architecture which is more or less well differentiated. While the development of adenocarcinomas of diffuse type is brutal, without frontrunner precancerous lesions, development of intestinal type adenocarcinomas undergoes a cascade of well-characterised histological events, allowing one to anticipate its appearance when preneoplastic lesions are detectable. *H. pylori* infection causes a Th1-type immune response and gastritis. The consequence is an increase in apoptosis of gastric epithelial cells (atrophy) generating a compensatory cell hyperproliferation and an altered differentiation leading to metaplasia [16]. Production of cyclooxygenase 2 (COX2), NO synthase, reactive oxygen and nitrogen species following the infection combined with the Th1 immune response are sources of errors during mitosis and participate in the accumulation of mutations [27].

6 The Stem Cell Hypothesis

The role of bone marrow-derived mesenchymal stem cells (BMDCs) in the neoplastic process has been recently highlighted. These cells which are recruited into the gastric mucosa following chronic inflammation and epithelial damage induced by *H. pylori* infection appear to be involved in about a quarter of the cells found in preneoplastic lesions [28], suggesting that gastric carcinoma may originate from both local epithelial stem cells and BMDC. Once recruited, these cells home in the gastric mucosa and fuse with local gastric epithelial cells, bearing local stem cell failure and participating in tissue regeneration. The context of chronic infection and inflammation leads to an epithelial mesenchymal transition (EMT) and altered tissue regeneration and differentiation from both local epithelial stem cells and BMDC. EMT induces the emergence of CD44-positive cells possessing mesenchymal and stem cell properties, resulting in metaplastic and dysplastic lesions to give rise, after additional epigenetic and mutational events, to the emergence of cancer stem cells (CSC) and adenocarcinoma [6, 7, 28, 2].

7 Conclusion

The association and the causal relationship between *H. pylori* infection and gastric adenocarcinoma as well as gastric MALT lymphoma is clearly established. Environmental factors and host genetic factors play probably an important role. However, for gastric adenocarcinoma, bacterial factors play a key role in the cascade of events leading to cancer. The CagA protein, specific for *H. pylori* and having no homology to other known proteins, is now regarded as a true oncogene. Host factors involved in gastric MALT lymphoma pathogenesis have to be determined, and new animal models of this disease will help to better investigate the mechanisms of deregulation of the inflammatory response.

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