

# Recent advances in *Helicobacter pylori* infection in children: From the petri dish to the playground

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*Helicobacter pylori* infection is acquired in childhood, plays a causative role in chronic gastritis and peptic ulcer disease, and is associated with the development of gastric cancer. The present review focuses on recent advances in the scientific knowledge of *H pylori* infection in children, including clinical sequelae, diagnosis and treatment. In addition, recent insights regarding both bacterial and host factors that mediate human diseases associated with *H pylori* infection are discussed.

**Key words:** *Diagnosis; Helicobacter pylori; Pathogenesis; Treatment*

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**H***elicobacter pylori* infection is usually acquired in childhood. In a recent retrospective longitudinal cohort study of 224 children in the United States, the highest rates of *H pylori* seroconversion occurred before the age of 10 years, with a seroprevalence rate of 25% by the ages of 18 to 23 years (1). In certain pediatric populations, the prevalence of infection may be even higher. For example, in a First Nations community in Manitoba with a high seroprevalence rate of infection in adults, the prevalence of *H pylori* infection was assessed in 163 children by stool antigen immunoassay, and was found to be approximately 50% in children less than two years of age (2).

### CLINICAL SEQUELAE OF *H PYLORI* INFECTION

In comparison with adults, children infected with *H pylori* only rarely develop complications like peptic ulcer disease. On the other hand, it is unclear if the organism causes other illnesses in children.

#### Recurrent abdominal pain in children

Currently, there are no compelling data to support an association between *H pylori* infection and recurrent abdominal pain in children (3). Application of a rigorous epidemiological tool (Hill's criteria) to published studies found inconsistent results, no temporal relationship, no biologic plausibility and no supporting experimental evidence for a role of *H pylori* infection in this condition (4). The majority of case-control studies

### Nouvelles connaissances sur l'infection à *Helicobacter pylori* chez les enfants : de la boîte de Petri au terrain de jeu

L'infection à *Helicobacter pylori* s'acquiert pendant l'enfance, joue un rôle étiologique dans la gastrite chronique et l'ulcère gastro-duodéal et est associée au cancer de l'estomac. Le présent article porte principalement sur les connaissances scientifiques acquises récemment sur l'infection à *H pylori* chez les enfants, notamment sur le diagnostic, le traitement et les séquelles cliniques de la maladie. Il sera également question des nouvelles notions concernant les facteurs de médiation, propres à l'hôte et à la bactérie, des maladies associées à l'infection à *H pylori* chez l'homme.

report a similar prevalence of *H pylori* infection in children with and without functional abdominal pain, as defined by Apley's criteria (5). To date, treatment trials have been uncontrolled or methodologically flawed and, therefore, do not provide additional useful information.

#### Dyspepsia

Controversy exists regarding the presence of a causal relationship between *H pylori* infection and dyspepsia (6). In the Canadian Adult Dyspepsia Empiric Treatment-*H pylori* Positive (CADET-Hp) study, which was performed in the primary care setting, patients with uninvestigated dyspepsia who were *H pylori* positive and randomized to eradication therapy had a greater improvement in symptoms than did those in the placebo group (50% versus 36%) (7). A meta-analysis, published in 1999, of 23 eradication studies and five treatment studies found that there was a higher prevalence of *H pylori* infection in adults with epigastric pain compared with controls, as well as an improvement in symptoms following eradication therapy (8). In contrast, a more recent (2001) meta-analysis concluded that *H pylori* treatment did not result in improvement of symptoms (9). Methodological differences between these two meta-analyses might explain the discordant results. In a recent study of 16 children with abdominal pain and *H pylori* infection, eradication therapy resulted in improvement in dyspeptic symptoms (10). This was an uncon-

trolled study, however, which makes it difficult to draw conclusions. Overall, the current data do not support a major causal role for *H pylori* infection in either recurrent abdominal pain in children or nonulcer dyspepsia in adults.

#### Iron deficiency anemia

Emerging data have identified an association between *H pylori* infection and refractory iron deficiency anemia (3,11). Cases of iron deficiency anemia, unresponsive to iron therapy but resolving with the eradication of *H pylori*, have been reported both in children (12,13) and adults (14). In addition, several case-control studies support a role for *H pylori* infection in this condition. In comparison with aged-matched controls, *H pylori*-infected subjects have lower mean ferritin and iron levels (15,16). In addition, ferritin levels improve in patients who receive *H pylori* eradication therapy. For example, a recent study of adolescent female athletes identified an increased risk of iron deficiency anemia in those with *H pylori* infection (17). A subset of these iron-deficient adolescents who were *H pylori* positive received either antibiotics or iron. Improvement in hematological parameters was observed only in the adolescents in whom the organism was eradicated. In a double-blind trial of iron-deficient children in Korea, subjects were randomized to three treatment groups: iron and *H pylori* eradication therapy, iron placebo and eradication therapy, or iron and placebo eradication therapy (18). Following treatment, the hemoglobin improved in both groups of children who received eradication therapy, but did not improve in the group who received iron therapy without *H pylori* treatment.

Several mechanisms have been postulated for the *H pylori*-associated reduction in total body stores of iron (11). One possible explanation is gastrointestinal bleeding due to gastritis, erosions, or ulceration. Most studies, however, have not detected occult gastrointestinal blood loss in infected patients with anemia (3). Decreased iron absorption due to a reduction in gastric acid and ascorbic acid secretion has also been postulated (11). An additional mechanism involves scavenging of iron or heme by the organism. Recent studies indicate that the organism possesses several genes that are involved in the uptake and utilization of iron (19,20).

A significant body of evidence links iron deficiency anemia with impaired development in young children (21). Furthermore, these cognitive and psychomotor effects may be reversible with replenishment of iron stores. Increasing evidence supports a causal role for *H pylori* infection and refractory iron deficiency. Therefore, further research is needed to determine the implications of testing and treating for *H pylori* infection in this setting.

#### Gastric cancer

An additional area of concern in the pediatric age group is the association between *H pylori* infection and the development of gastric cancer in adulthood. The World Health Organization has classified *H pylori* as a carcinogen (22). Epidemiological data indicate that *H pylori* infection increases the risk of gastric cancer up to four-fold (23). In a recent prospective study, 1526 Japanese subjects, of whom 1246 had *H pylori* infection, were followed for a mean of 7.8 years (24). Gastric cancer developed in patients with *H pylori* infection, but not in the uninfected group. It remains unknown whether *H pylori* eradication therapy can prevent the development of gastric malignancies, but the progression of gastric atrophy, which is a precancerous

lesion, can be halted by such therapy (25). In a comprehensive review of the literature, Imrie and colleagues (26) suggested that screening and treatment for *H pylori* infection in children, in an attempt to prevent gastric cancer, is not warranted.

In summary, recent data show that *H pylori* infection in childhood is associated with conditions other than peptic ulcer disease, such as refractory iron-deficiency anemia. *H pylori* infection has also been purported to cause a wide variety of other extraintestinal manifestations, such as dermatological conditions, sudden infant death syndrome and short stature (3). However, the evidence supporting these associations is not compelling.

#### DIAGNOSIS OF *H PYLORI* INFECTION

North American and European guidelines for the diagnosis and treatment of *H pylori* infection in children have been developed (27-29). Each of these consensus statements recommends upper endoscopy for the detection of *H pylori* infection in children over 'test and treat' strategies. Nevertheless, large-scale epidemiological studies are clearly warranted to enhance our knowledge of *H pylori* infection in children. Therefore, accurate noninvasive methods for *H pylori* detection in this age group are essential.

Serological tests for *H pylori* antibodies are readily available, inexpensive and easy to perform. However, because the sensitivity of these tests in children is much lower than in adults, serologic testing for *H pylori* is not recommended (30,31).

The urea breath test is a noninvasive test that detects the presence of urease-producing organisms like *H pylori*. In older children, this test compares well with the gold standard tests, endoscopy and histology (32-34). The reliability of urea breath testing in very young children, however, has been questioned (35). The test is more difficult to perform in this age group and yields a high rate of false-positive results (36,37). For example, in a recent study of 149 German children, a high false-positive rate was documented in subjects less than six years of age (36). Similarly, Imrie and colleagues (37) found that the positive predictive value of the test was 80% in Irish children over the age of two years but was only 50% in younger children. They also found that 12.5% of 72 children under the age of four years were unable to provide adequate breath samples for analysis.

If appropriately validated, stool antigen testing may well prove to be a suitable alternative for pediatric patients (30). Studies performed to date in this age group report sensitivities and specificities of greater than 90% when compared with the gold standards of histology, rapid urease testing and bacterial culture (38-41).

#### TREATMENT OF *H PYLORI* IN CHILDREN

The optimal therapy for the eradication of *H pylori* infection in children is not known. The recommendations for eradication therapy outlined in consensus statements are based on extrapolation of data derived from randomized controlled trials in adults, because the majority of pediatric studies are small open-label trials (42). A recent paper, however, reports the results of a multicentre prospective randomized double-blind controlled trial comparing two *H pylori* eradication therapies triple therapy with omeprazole, amoxicillin and clarithromycin versus dual therapy with amoxicillin and clarithromycin in a pediatric population (43). In intention-to-treat analysis, the eradication rate with triple therapy was 74.2%, compared with only

9.4% in the dual therapy group ( $P < 0.01$ ). Thus, the success rate with triple therapy was lower than that found in previous studies of adult patients, and was less than the 80% cure rate considered acceptable for adults (44). Poor compliance, rather than antibiotic resistance, may have been an explanation for the lower cure rate in this population. Further studies employing other drugs and different dosage schedules should be undertaken in an attempt to improve upon the standard triple therapy regimen.

## PATHOGENESIS OF *H PYLORI* INFECTION

### Virulence factors

Since only a minority of *H pylori*-infected subjects develop complications associated with the infection, such as peptic ulcer disease or gastric cancer, the mechanisms underlying *H pylori*-mediated disease continue to be under intense investigation. Both bacterial and host factors contribute to the pathogenesis of *H pylori* infection. What follows is a description of recent insights into these factors and their contributions to the disease.

### Bacterial factors

**Colonization:** Urease may be essential both for the survival of *H pylori* in the local acidic environment of the stomach and for promoting bacterial colonization. This conclusion is supported by the observation that urease-mutant strains fail to colonize gnotobiotic piglets (45). Urease activity is regulated by a unique pH-gated urea channel, termed UreI, which is encoded by the *ureI* gene. The UreI channel is open at low pH, thereby promoting the entry of urea, which buffers the acidic pH. At neutral pH, on the other hand, the urea channel closes and shuts down the influx of urea (46). Deletion of *ureI* abolishes urease activity and bacterial resistance to gastric acid (46,47).

The binding of *H pylori* to epithelial cells is facilitated by the actions of several bacterial-surface components, known as adhesins (48). The best-characterized of these is the blood group antigen binding adhesin, BabA2, which binds to the Lewis B receptor on gastric epithelial cells (49). Patients harboring strains possessing the *babA2* gene exhibited higher levels of bacterial colonization, neutrophil infiltration and interleukin (IL)-8 mRNA in gastric mucosa compared with patients infected with *babA2*-negative strains (50). These findings suggest that BabA2 facilitates bacterial colonization and augments host immune responses. In addition, some studies have found that infection with *babA2*-positive strains is associated with peptic ulcer disease (51) and preneoplastic gastric lesions (52). More recently, a novel adhesin on *H pylori*, termed SabA (for sialyl-dimeric-Lewis X antigen binding adhesin), has been identified (53). SabA binds the corresponding receptor, sialyl-dimeric-Lewis X (sLe<sup>x</sup>), which is present in gastric epithelial cells. Gastric tissue inflammation and malignant transformation each promote synthesis of sialylated glycoconjugates, which are rarely found in healthy gastric tissue (54). Chronic *H pylori* infection also up-regulates expression of sLe<sup>x</sup> antigens, which *H pylori* in turn can exploit for adherence to the surface epithelium (53). Mahdavi and colleagues (53) suggested that *H pylori* uses BabA2 for strong and specific recognition of the receptor for Lewis B antigen on gastric epithelial cells. During persistent infection and chronic inflammation, *H pylori* triggers up-regulation of the inflammation-associated sLe<sup>x</sup> antigen, allowing SabA to bind its corresponding receptor, sLe<sup>x</sup>.

### Type IV secretion system and the *cag* pathogenicity island:

Certain *H pylori* strains express two independent type IV secretion systems (55). *H pylori* employs a 'classical' type IV secretion system that is encoded by genes on the *cag* pathogenicity island (PAI), a 37-kb genomic fragment containing 29 genes (56). In addition, ComB proteins encoded by *comB* genes have all the characteristics of a pore-forming transmembrane complex that is suitable for the translocation of DNA through the cellular envelope (57). The *cag* type IV secretion system and the ComB system are functionally unrelated. Mutation in the ComB operon does not affect the function of *cag*, and deletion of *cag* PAI does not result in decreased transformation efficiency (57).

The *H pylori* cytotoxin-associated gene (*cagA*) is a marker for *cag* PAI. A number of independent groups have shown that CagA is translocated into epithelial cells and phagocytes via the type IV secretion system (58-60). Following translocation, CagA is tyrosine-phosphorylated by a host Src kinase and induces a growth factor-like (hummingbird) phenotype (61). The Src homology 2 domain-containing tyrosine phosphatase (SHP-2) has recently been identified as the intracellular target of CagA (62). Formation of a CagA-SHP-2 complex is an essential prerequisite for induction of the 'hummingbird' phenotype.

**Vacuolating cytotoxin:** The role of vacuolating cytotoxin (VacA) in mediating disease is unclear (63). This protein was originally named for its ability to induce vacuolation in tissue culture cells. Recent evidence suggests that both recombinant and native VacA induce apoptosis in a gastric epithelial cell line in vitro (64). VacA targets the mitochondrial membrane and induces cytochrome C release, leading to programmed cell death (65). Infection of polarized monolayers with an *H pylori* strain expressing VacA induces a decrease in trans-epithelial resistance across the cell monolayer, whereas infection with an isogenic *vacA* mutant strain does not lower the trans-epithelial resistance, suggesting that VacA is able to increase the paracellular permeability of the gastric epithelium (66). Using a gastric epithelial cell monolayer wound model, Pai and colleagues (67) demonstrated that VacA treatment significantly inhibits wound re-epithelialization and cell proliferation. These findings suggest that VacA might interfere with the repair of gastric mucosal injury and ulcer re-epithelialization. A recent study demonstrated that VacA behaves as a low pH-activated, passive urea transporter that increases the transepithelial flux of urea (68). Therefore, VacA might provide the bacterium with a competitive advantage by increasing the availability of urea. In support of this concept, VacA has been shown to be important for initial colonization in a murine model of *H pylori* infection (69).

The *oipA* (outer inflammatory protein) gene encodes one of the outer membrane proteins, and is a putative virulence factor located approximately 100 kb from the *cag* PAI on the *H pylori* chromosome (70). By performing a backward stepwise multiple regression analysis, Yamaoka and colleagues (71) demonstrated that among *cag* PAI, *vacA*, *babA2*, *iceA* and *oipA* in *H pylori* strains isolated from 247 patients, only the presence of functional *oipA* was able to distinguish infected patients with duodenal ulcer disease from those with gastritis only. The presence of functional *oipA* was also associated with increased *H pylori* density, enhanced neutrophil infiltration and elevated mucosal IL-8 levels, but was not correlated with gastric atrophic changes. Further studies are required to confirm these findings in other populations.

**Lewis blood group antigens:** *H pylori* strains from North American and European populations mainly express type 2 Lewis blood group antigens (Le<sup>x</sup> and Le<sup>y</sup>) (72), but those isolated from Asian hosts appear to be more likely to also express type 1 Lewis antigens (Le<sup>a</sup> and Le<sup>b</sup>) (73,74). In some populations, peptic ulcer disease appears to be associated with increased expression by *H pylori* of Lewis antigens (73,75). Lewis antigen expression among *H pylori* isolates from asymptomatic individuals is characterized by a relative absence of type 1 Lewis antigens (Le<sup>a</sup> and Le<sup>b</sup>), a decrease in Le<sup>x</sup> expression, and an increase in nontypeable *H pylori* (76,77). Monteiro and colleagues (78), studying organisms isolated from asymptomatic subjects, reported that *H pylori* strains that were not typeable using monoclonal antibodies specific for Lewis antigen (Le<sup>a</sup>, Le<sup>b</sup>, Le<sup>x</sup>, or Le<sup>y</sup>) produced lipopolysaccharide molecules devoid of 'blood-group O-chain' regions. These studies suggest that Lewis antigens might be important to the pathogenesis of *H pylori* infection.

The role of Le<sup>x</sup> in bacterial adhesion remains controversial (79,80). For example, in a mouse model, Takata and colleagues (80) demonstrated that both wild-type strains expressing Le<sup>x</sup> and Le<sup>y</sup>, as well as isogenic mutant strains that do not express Le<sup>x</sup> or Le<sup>y</sup>, colonize the mouse stomach to a similar degree. In contrast, another study demonstrated that a Le<sup>x</sup> mutant strain does not adhere to human gastric mucosal tissue, whereas the Le<sup>x</sup>-positive parent strain adheres well (79). An additional study in humans showed that *H pylori* strains that strongly express Le<sup>x/y</sup> are associated with a higher colonization density in gastric tissues obtained from human subjects compared with strains that weakly express Le<sup>x/y</sup> (75).

### Host factors

**Immune responses:** Despite the presence of a vigorous immune response, *H pylori* eradication is not observed unless specific antibiotic therapy is provided. This finding suggests that *H pylori* is able to evade the host immune response. Phagocytosis is an essential component of the innate immune system and certain pathogens like *Mycobacterium*, *Shigella*, and *Salmonella* have evolved strategies to avoid being killed and degraded within phagolysosomes (81).

Recent evidence suggests that *H pylori* also disrupts the normal phagocytic process. Allen and colleagues demonstrated that type 1 *H pylori* strains, which possess *cag* PAI and produce VacA, exhibit delayed phagocytosis in association with the formation of large phagosomes, termed 'megosomes' (82). Another study provided evidence that *cag* PAI-positive *H pylori* strains resist phagocytosis (83). In contrast, Odenbreit and colleagues (60) found no difference in the phagocytosis of type 1 and type 2 strains. A recent study demonstrated that both type 1 and type 2 *H pylori* strains are capable of subverting bacterial killing by macrophages for up to 24 h (84). In comparison with type 2 strains, however, survival of *H pylori* strains expressing VacA was enhanced in association with prevention of phagosome maturation and retention of TACO protein (coronin 1). TACO, a homologue of *Dictyostelium* coronin, transiently associates with phagosomes during phagocytosis. During uptake of *Mycobacterium bovis*, however, TACO is actively recruited and retained, thereby preventing phagosome maturation and resulting in bacterial survival (85). Similarly, retention of TACO by *H pylori* strains expressing VacA might disrupt phagosome maturation.

**T-helper 1/T-helper 2 paradigm:** CD4<sup>+</sup>-naïve T cells can be

divided into two functional subsets, T-helper 1 (Th1) and T-helper 2 (Th2) cells (86). Th1 cells secrete IL-2 and interferon-gamma (IFN- $\gamma$ ), and induce cell-mediated immune responses that regulate the resolution of infection with intracellular pathogens. Th2 cells produce IL-4, IL-5, IL-6, and IL-10, and promote humoral-mediated immune responses generally involved in resolving infection with extracellular pathogens.

The role of Th1/Th2 in the pathogenesis of helicobacter infection is controversial (87-89). In a murine model of infection, *H pylori*-infected severe combined immunodeficiency mice reconstituted with splenocytes from IL-10-deficient mice developed more severe gastritis than mice reconstituted with splenocytes from wild-type mice. In contrast, mice reconstituted with IFN- $\gamma$ -/- splenocytes developed less severe gastritis during infection in comparison with mice reconstituted with wild-type splenocytes (89). These findings suggest that IFN- $\gamma$  produced by Th1 cells contributes to gastritis, whereas IL-10 produced by Th-2 cells or T regulatory cells suppresses inflammation. Infection of mice with a replication-defective adenovirus during chronic infection with *Helicobacter felis* led to a significant decrease in *H felis* colonization in an IFN- $\gamma$ - and IL-12-dependent manner (ie, one that depends on Th1 cytokines). This finding supports a role for Th1 responses in controlling helicobacter infection (88). Similarly, infection with *H felis* and a murine nematode reduces Th1 cytokine responses, compared with infection with *H felis* only, and enhances Th2 responses in association with a decrease in gastric inflammation and atrophic gastritis (90). This finding indicates that coinfection with a nematode can modulate host immune responses, thereby altering the disease outcome.

These results have been implicated in explaining the 'African enigma': despite a high prevalence of *H pylori* infection in Africa, the prevalence of gastric cancer is extremely low (91). A recent study supports the notion that the host immune response to *H pylori* in an African population differs to that in subjects from developed countries (92). This study found that 81% of infected adults and 90% of infected children in an African population exhibited a Th2-predominant response (with Immunoglobulin G1), as opposed to a Th1-predominant response (with Immunoglobulin G2), whereas this was found in only 4.7% of Australian and 4.4% of German subjects.

**Interleukin-1 beta gene:** Gastric cancer and duodenal ulceration are mutually exclusive outcomes of *H pylori* infection. Nevertheless, various *H pylori* strains are equally associated with both diseases, which indicates that host factors might play a role. El-Omar and colleagues (93) provided evidence that individuals with specific polymorphisms of the interleukin-1 beta (IL-1 $\beta$ ) gene cluster, which are associated with enhanced IL-1 $\beta$  production, are at increased risk for the development of corpus gastritis, hypochlorhydria and gastric atrophy following *H pylori* infection. These findings have been confirmed in other populations. For example, a recent study in Japan demonstrated that *H pylori*-infected individuals that possess these proinflammatory IL-1 $\beta$  polymorphisms have an increased risk of hypochlorhydria and atrophic gastritis and a reduced risk of duodenal ulceration (94). These polymorphisms can also predispose to the development of gastric cancer, which is associated with atrophic gastritis (95,96).

**Gastric epithelial cell apoptosis:** *H pylori* induces apoptosis of gastric epithelial cells both in vitro and in vivo (97). Current evidence suggests that several mechanisms are involved. For

example, a recent study suggests that *H pylori* is capable of directly inducing apoptosis in epithelial cells in vitro mainly by releasing cytochrome C from mitochondria (98). Host immune responses may also mediate apoptosis during infection. Cytokines produced by Th1 cells, such as tumour necrosis factor alpha (TNF- $\alpha$ ) and IFN- $\gamma$ , markedly potentiate *H pylori*-induced apoptosis in gastric epithelial cells, whereas those produced by Th2 or Tr1 cells, such as IL-10, inhibit it (99). Furthermore, expression of TNF- $\alpha$  and IFN- $\gamma$  in situ correlates with epithelial cell apoptosis and gastritis during *H pylori* infection in vivo (100).

In addition, the Fas-Fas ligand system is also involved in *H pylori*-triggered apoptosis. Infection of Fas-deficient mice with *H pylori* (101) or *H felis* (102) is associated with less than the usual degree of gastric epithelial cell apoptosis. Furthermore, absence of Fas signaling during *H pylori* infection enhances premalignant gastric mucosal changes (101). It is

therefore possible that *H pylori*-induced apoptosis plays a role in the development of gastric cancer. Scotiniotis and colleagues (103) demonstrated that *H pylori*-induced apoptosis is associated with increased epithelial proliferation in the absence of premalignant lesions or gastric cancer; however, in the presence of intestinal metaplasia, apoptosis reverts to normal levels whereas cell proliferation remains increased. This leads to the hypothesis that alterations in cell turnover during *H pylori* infection might play a key role in gastric carcinogenesis (99).

## CONCLUSION

Investigation of *H pylori* infection in children continues to provide useful insights into the natural history of the disease. Although substantial progress has been made in our understanding of the pathogenesis of *H pylori* infection, important questions remain regarding host-bacterial interactions.

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