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Pathobiology of *Helicobacter pylori* Infection

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INTRODUCTION

The extraordinarily high world-wide prevalence of *Helicobacter pylori* infection has become apparent over the last decade. Its associated disorders are becoming clearer and revealing some fascinating paradoxes. For example, duodenal ulcer has been shown to be "caused" by *H. pylori*, at least by the near absence of recurrence after eradication of the infection. Similarly, gastric adenocarcinoma has been strongly associated with *H. pylori* infection, but herein lies the paradox, in that, for decades, it has been known that the development of a duodenal ulcer is protective of the development of gastric cancer. This has been formally established by Parsonnet and co-workers who demonstrated a negative correlation between the two disorders [1]. This raises the obvious question as to what pathogenic mechanisms underlie the evolution of such widely divergent clinical events. Another seeming paradox concerns the development of duodenal ulcer by *H. pylori*. Duodenal ulcer has generally been associated with an increase in gastric acid secretion. However, upon eradication of *H. pylori*, basal acid secretion returns to normal levels, but peak acid output remains elevated for some time. Does this mean that peak acid secretion is genotypically determined, or is it a consequence of an increased parietal cell mass induced by a long-term *H. pylori* infection? Further confusion has been engendered by an early observation on the natural history of peptic ulcer disease by a general practitioner in England, who despite the obvious limitations of his contemporary diagnostic techniques, showed that peptic ulcer disease "burns out" over time [2]. Does that mean that gastric atrophy reduces secretion of acid to the point that Schwartz's dictum of "no acid, no ulcer" becomes applicable?

This paper will review some of the interactions between *H. pylori* and the gastric epithelium that have been studied, to the point that some clues can be obtained as to how this bacterium can evoke such an extraordinary range of physiological responses with their ensuant pathological processes. Clearly, we are only at the very beginning of an understanding. Much of what was written on gastric physiology and pathology before Marshall and Warren's seminal paper [3] needs to be read with caution, in that disease groupings or physiological responses may need to be reconsidered based on the presence or absence of *H. pylori*. Perhaps the oldest example of this problem is that of the gastric urease that was thought to be a gastric epithelial enzyme until the discovery of *H. pylori* [4]. Even now, the role of the urease is not clear. Its site of localization remains controversial; does it remain intracytoplasmic until it is released on death of the organism or is it actively secreted into the gastric environment [5]? We now have the technology to begin to address these questions, but ironically in the developed world, the prevalence of new cases of *H. pylori* infection has plummeted [6]. Treatment is becoming very effective thus making it difficult for investigators to have access to required clinical material.

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[b]*Abbreviations:* PCR, polymerase chain reaction; GRP, gastrin releasing peptide; GERD, gastroesophageal reflux disease; MAG, multifocal atrophic gastritis.

We will discuss the pathobiology of *H. pylori* infection chronologically by arbitrarily dividing the natural history of infection into the early, middle and late phases. Certainly there is overlap between these phases, but this structure does provide a framework for discussion.

EARLY EVENTS

The natural history of *H. pylori* infection is poorly defined. Much of the work on this persistent or "slow infection" [7] is based on extrapolation perforced by the difficulties of making serial observations on an organism whose natural habitat is difficult to access. Most observations have been made of single points in time without any real knowledge of when infection was initiated. Serial observations are comparatively rare. This then makes interpretation difficult when the investigator is trying to relate that observation to an infection that may be life-long.

The earliest events of *H. pylori* infection are quite well described from a series of observations gleaned from volunteers who ingested the organism and from careful observation of research volunteers who were inadvertently infected with *H. pylori*. There are no data in young children, which is the predominant acquisition time of infection, except for the first report of acute acquired achlorhydria by William Osler [8]. Although in early childhood there is evidence for infection, loss of infection and further reinfection [9-11], we know little or nothing about the details of these events. Thus adult infection is the exception, and we can, for now, only assume that it parallels the events in children.

An oriental volunteer was closely monitored during a study of the effects of aspirin on the gastric mucosa [12]. He appeared to be infected with *H. pylori* during an endoscopic examination of his stomach. In the first few days, he was shown to have an increase in mucus output, blood loss and DNA release into the gastric lumen. By day eight, he had become profoundly achlorhydric, and an acute gastritis had developed. He remained achlorhydric for two months, but over time, he spontaneously recovered his acid secretion. Marshall voluntarily confirmed Koch's postulates and noted the development of gastritis within a few days of ingesting 1×10^9 c.f.u of *H. pylori* [13]. The gastritis was notable for the predominance of polymorphonuclear leukocytes. Subsequently, Morris repeated this experiment in more detail [14]. He initiated infection with 3×10^5 c.f.u. of *H. pylori*, having failed to induce infection with the same strain that Marshall had ingested. He too developed a pangastritis with the predominance of polymorphonuclear white blood cells, and eight days after infection developed achlorhydria. Both conditions persisted until day 26 when he took doxycycline. By day 29, his acid production returned to normal, but the infection and the gastritis persisted.

A larger epidemic of retrospectively diagnosed *H. pylori* infection thought to have been transmitted by a contaminated pH probe has provided further useful data. Of 37 volunteers who participated in a gastric acid secretion study, 17 developed achlorhydria and gastritis [15]. Profound achlorhydria was noted three to 75 days after the onset of clinical symptoms with a maximal decrease of peak acid output occurring at a mean of 25 days (range seven to 49 days) after infection. During this period, a severe fundic gastritis was noted in all those who were achlorhydric, though parietal cells were noted to be morphologically intact. Despite severe neutrophilic gastritis, gastric permeability remained normal. Pepsinogen 1 levels were substantially elevated but returned to normal levels. Gastrin levels were elevated but were similar both during hypochlorhydria and subsequently. Of particular note is that three of the seventeen volunteers did not experience a return of acid secretion over 12 months of follow-up. In addition, a patient with Zollinger-Ellison syndrome became achlorhydric at the same time and remained so from 1976 to 1978.

Other observations of achlorhydria during the early stages of infection include a small epidemic described by Gledhill and co-workers [16]. A number of isolated occurrences of achlorhydria have been reported, often in association with contaminated instrumentation. An additional case of achlorhydria in another case of Zollinger-Ellison syndrome was reported with prolonged and carefully studied acid secretion [17]. In a study designed to investigate the prevalence of *H. pylori* in healthy asymptomatic volunteers, 25 percent of those who were *H. pylori* positive were found to be achlorhydric [18].

Animal studies with various *Helicobacter* species have been reported in which there was an *in vivo* correlation with the human studies in that reduced acid secretion appeared to be an early event. Fox et al. demonstrated the elevation of gastric pH in ferrets within a few weeks of infection with *H. mustelae* [19]. A study in beagles showed elevations in gastric pH in several animals within days after infection with *H. felis* [20]. No systematic acid secretory studies have been reported using the non-human primate model, the mouse model or the gnotobiotic pig. The analogous *in vitro* experiments have been performed using *H. pylori*, *H. felis* and *H. mustelae* on rabbit and ferret cells, respectively [21]. Experiments using guinea pig parietal cells and *H. pylori* yielded similar results [22], as did the use of *H. pylori* and human-derived gastric glands [23].

Thus, early on after infection with *H. pylori*, at least in adults and probably in children, there is a series of events that occur that profoundly alters gastric physiology. In particular, there is a severe fundic gastritis associated with an overt neutrophilic infiltrate and heavy colonization with bacteria. This is followed by the profound loss of acid secretion that spontaneously recovers in most, but not all individuals. The gastritis appears to start before achlorhydria develops, and thus, the reduced acid secretion is not part of the initial colonization process. There is the possibility that this period represents a phase during which excretion of *H. pylori* can occur as has been demonstrated for *H. mustelae*. This organism has been shown to be easily detectable in ferret feces during periods of achlorhydria induced by either *H. mustelae* or by omeprazole [24]. No such data has been reported in man other than two reports of the successful isolation of *H. pylori* from the feces of children in the Gambia and from dyspeptic adults in the United Kingdom [11, 25]. Even the use of the highly sensitive polymerase chain reaction (PCR)[b] has been inconclusive in demonstrating *H. pylori* in human feces [26].

MIDDLE PERIOD

Once infection with *H. pylori* has been initiated and gastritis is established, a number of options appear possible. First, in the majority of individuals, the gastritis slowly evolves with a reduction but not total loss of the neutrophilic infiltrate. The gastritis converts from the acute pangastritis to a predominantly antral gastritis with lymphocytic, predominantly Th1 cells and monocytic infiltration. In the majority of those infected, this process probably does not progress further, and a symbiotic relationship develops. There are no known indicators of how long an individual has been infected with *H. pylori*. The second possibility is the development of duodenal ulcer disease, generally considered to be a condition of increased acid secretion. The third alternative is for gastric ulceration to develop, generally noted to be associated with normal to reduced acid secretion. The fourth alternative is of the uncommon event of persistent hypochlorhydria. Long-term hypochlorhydria may be present in the United Kingdom in five percent of those individuals infected with *H. pylori* [27]. Finally, infection could terminate either spontaneously by mechanisms unknown or inadvertently with the use of antimicrobial agents [28]. Which course an individual will follow is at present unpredictable given our limited knowledge of the virulence factors related to *H. pylori*. Our knowledge of the host factors involved is even

more limited. However, certain observations have been made with regard to physiological processes that occur in each group.

In the asymptomatic *H. pylori* infected individual, the gastritis is predominantly antral in location. This gastritis is associated, in turn, with a reduction in the number of D cells that produce somatostatin. G cells appear unaffected, and there is wide agreement that gastrin levels are generally increased [29]. Basal acid secretion and post-prandial acid secretion do not appear to be very different between those who are infected and those who are not. However, gastrin releasing polypeptide (GRP)-stimulated acid secretion is increased three-fold along with a comparable increase in gastrin release in those infected with *H. pylori* [30].

The second scenario applies to those individuals who develop duodenal ulcer disease in response to *H. pylori* infection. No data have been reported on the relationship of age of infection to the development of ulceration. This point of ignorance has two ramifications: First, we are unlikely to ever rigorously define this relationship, because the invasive nature of diagnostic studies precludes frequent observations. Second, this is a significant pitfall in terms of case selection for studies designed to compare differences between those merely infected with *H. pylori* and those with ulcers. At present there is no way of defining whether or not an individual infected with *H. pylori* may suddenly develop an ulcer.

The pathophysiology of *H. pylori*-induced peptic ulcer disease is not well understood. The traditional view that ulcerogenesis occurs when mucosal defensive mechanisms become overwhelmed by aggressive luminal factors remains a useful paradigm. *H. pylori* may promote ulcer development directly, for instance, by elaborating a vacuolating cytotoxin, or indirectly by increasing acid secretion (see below). One novel hypothesis is that *H. pylori* damages the gastroduodenal mucosa by promoting epithelial cell apoptosis [31]. Recent data have also been developed indicating that *H. pylori* may inhibit mucosal bicarbonate secretion, an intrinsic defense mechanism of gastric and duodenal mucosa [10]. This area of research is rapidly evolving, and many of the current possibilities have been reviewed [32-34].

Several other recent observation are worth reviewing. Duodenal ulcer is generally associated with an increase in acid secretion. Until recently, this was assumed to be due to an increase in parietal cell mass [35] that, in turn, was assumed to be genetically determined. Three recent observations have been made that on eradication of *H. pylori* peak acid output recovers to normal values after a six to 12 month period [36-38]. These observations can be interpreted as follows: Peak acid secretion, a function of parietal cell mass, is not only genetically determined but on long-term infection with *H. pylori* becomes increased [37]. The slow reduction of peak acid secretion after *H. pylori* eradication may reflect the relatively slow turnover of parietal cells in man.

El-Omar has shown that in duodenal ulcer patients infected with *H. pylori* the use of intravenous GRP results in a three-fold increase in gastrin release and a six-fold increase in acid secretion [30]. This was interpreted as an increased sensitivity of the parietal cell by mechanisms unknown. The effect of GRP on basal secretion disappeared 12 months after eradication of *H. pylori*, but there remained a significantly elevated response to gastrin 17 at 12 months [36]. This finding remains unexplained. In a study by Labenz et al., [39] it was shown that both before and after treatment of *H. pylori* in those with duodenal ulcer, 24-hour median gastric pH was about the same, 1.0 and 1.1, respectively. However, the effect of omeprazole in 17 patients before and after eradication of *H. pylori* revealed a surprising difference in gastric pH. Omeprazole was significantly less effective after *H. pylori* eradication therapy as assessed by 24-hour intragastric pH measurements. Median pH went from 5.5 to 3.3 after eradication, superficially a striking change, but it should be remembered that the reduction from pH 1 to 3.3 is a more than a 99 percent reduction in

H^+ ion concentration. Gillen and McColl (in press, Gut) have performed similar experiments in *H. pylori* positive patients without ulcers and have demonstrated a more dramatic effect still by measuring gastric acid secretion. In omeprazole-treated individuals, acid secretion was effectively nil when *H. pylori* was present, and when the infection was cured, secretion was significantly increased. Further support for this finding comes from observations made in patients with Zollinger-Ellison syndrome. Not only do patients with this syndrome who are *H. pylori* positive require less proton pump inhibitor therapy, but their stimulated acid secretion is only about 60 percent that of those infected with *H. pylori*, mean maximal acid output being about 40 and 66 mEq/hour, respectively [40]. An explanation for these findings is not readily apparent. The data are consistent with the possibility that *H. pylori* produces an acid inhibitory factor that works synergistically with omeprazole. It is not known if this effect is specific for omeprazole or whether it is a generic property of proton pump inhibitors. An earlier study comparing the effect of omeprazole and lanzoprazole reported comparable efficacy for both with median 24 hour pH values of 5.5 and 5.4, respectively [41]. Post *H. pylori* treatment studies were not conducted, but the placebo response was a pH of 1.3. This hypothesis is also consistent with earlier observations that omeprazole in volunteers had little antisecretory activity at a dose of 10 mg, but in ulcer patients there was some clinical activity [18].

The enhanced efficacy of omeprazole in *H. pylori* positive patients should be studied further for two reasons. There has been concern regarding the possibility of accelerated gastric atrophy in patients with gastroesophageal reflux disease (GERD) who require long-term omeprazole therapy and also are infected with *H. pylori* [42]. A recent FDA panel concluded that the issue was not substantiated. However, the treatment of *H. pylori* may render the control of the GERD more difficult. This is a novel therapeutic dilemma. Furthermore, there is early evidence that some patients with duodenal ulcer who are treated successfully for *H. pylori* subsequently develop symptoms of GERD. This raises the interesting philosophical issue as to whether this pathogenic organism, found on the gastric mucosa of greater that 60 percent of the world's population, may also have some beneficial role. This is an ironic twist in that omeprazole has become a major component of eradication therapy for *H. pylori*. A further surprise was reported by Banerjee et al. [43] who found that *H. pylori* was suppressed by sucralfate, an aluminum sucrose polymer. This suppression was associated with a reduction of GRP-stimulated gastrin secretion and acid secretion. Comparable studies in *H. pylori* associated gastric ulcers have not been performed.

Other secretory processes in the gastric epithelium have received much less scrutiny. Gastric juice vitamin C levels are now well known to be reduced in *H. pylori* infection, though serum levels of this vitamin remain normal [44]. This may be due, in part, to a bacterial oxidase, but there may also be an impairment of secretion secondary to the gastritis [44, 45]. Eradication of the organism results in normalization of luminal vitamin C levels. Pepsinogen levels are elevated by *H. pylori* infection, particularly during the acute stage. Initially, the change in the various pepsinogens was thought to be a genetically determined event and was used as a marker for duodenal ulcer disease [46]. However, the patterns returned to normal after eradication of *H. pylori* [47, 38]. Subsequently there have been suggestions that pepsin release is mediated by a small molecule produced by *H. pylori* [48] or by bacterial lipopolysaccharide [49].

LATE PERIOD

The concept of a late period of infection is obviously arbitrary. However it does serve to conceptualize several important issues. The vast majority of those infected with *H.*

pylori continue to carry their infection with no ill effects. There is some evidence for spontaneous loss of infection, but whether this is truly spontaneous or a loss facilitated by the incidental use of antibiotics is unknown. Acid secretion in the elderly continues in most individuals unabated. Pepsinogen levels may fall. Duodenal and gastric ulcers are well known to occur late into life, but are comparatively uncommon.

Atrophy of the gastric mucosa occurs in some individuals over time. What actually constitutes atrophy is difficult to define. In pernicious anemia, it is comparatively easy with complete loss of specialized elements such as chief and parietal cells. Is, however, an intensely inflamed antrum with apparent loss of glandular elements the same as the loss of glandular elements in the gastric body with little inflammation? Correa, in 1984, initiated and has popularized the concept of multifocal atrophic gastritis (MAG) [50]. This entity appears to start in the area of the incisura and spread in a patchy manner both proximally into the body and distally into the antral mucosa. However, there are now questions regarding the relationship of antral gastritis to MAG. This condition is regarded as one of the phases in the development of gastric neoplasia along with the development of intestinal metaplasia [51].

Little is known of the secretory capacity of the stomach during a particular type and degree of mucosal atrophy. A recent study suggests that at least some atrophy is functionally reversible after eradication of *H. pylori* [52]. Of particular note is a recent observation, albeit in mice, that atrophy is a function of the host and not that of the infection. Large differences in atrophy were observed in mice of different genotypes infected with the same strain of *H. felis* or *H. pylori*. Furthermore, there was poor correlation between the degree of bacterial colonization and the site of atrophy. For example, colonization of the antrum in C57BL/6 mice at two months was associated with severe body atrophy at six months [53]. The other issue relating to atrophy is the observation by Fry that the recurrence of ulcer disease tends to spontaneously enter remission after reaching a peak period [2]. His observations on the natural history of ulcer disease, which for obvious reasons cannot be repeated, raise the possibility that atrophy can occur in those who initially developed ulcer disease. Some support for this comes from the observation that estimates of the parietal cell populations on the margins of partial gastrectomy specimens, resected for gastric neoplasia or benign conditions, are almost identical [54]. Sipponen, in a recent review [55], suggests that in those who hypersecrete acid, gastritis and atrophy predominate in the antrum and that those who are hyposecreters tend to have atrophy of the corpus. Clearly, what is needed is much more data on the structure-function relationships between the various anatomical patterns of gastritis and the amount of acid secreted.

CONCLUSION

We conclude with the following hypothesis for the pathobiology of *H. pylori* infection. On initial infection, the body of the stomach becomes acutely inflamed and acid secretion is inhibited. The inflammation is slowly modulated and the organism migrates to the antrum. Those who develop acid hypersecretion may develop duodenal ulcers; the majority develop a symbiotic relationship, and a few with predominant corpus infection have normal to reduced acid secretion and develop gastric ulcers. A small number of hyposecreters with mucosal atrophy develop carcinoma. The use of proton pump inhibitors has revealed an unexpected degree of latent acid inhibitory activity associated with *H. pylori* that is related, at least in part, to colonization of the corpus. In other words, there is a local effect of *H. pylori* on the parietal cell. Whether this effect is mediated by ammonia or by some other specific bacterial product is not known.

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