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REVIEW ARTICLE

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Helicobacter pylori: the new bug on the (paraffin) block

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Abstract A nameless spirillar organism in search of a disease only a few years ago, *Helicobacter pylori* has seen its fortunes suddenly reversed. After a rapid succession of name changes and some acrimonious disputes across continents, in less than a decade *H. pylori* has been catapulted to the centre stage of gastroenterological and microbiological research and has topped the most-wanted list of the pharmaceutical industry. The discovery of *H. pylori* has provided the momentum for the formation of the group that eventually created the Sydney System. Today, *H. pylori* is increasingly recognized as one of the most prevalent human pathogens worldwide. Its causal association with chronic active gastritis is undisputed and its role in the pathogenesis of peptide ulcer disease, although still poorly understood, is universally accepted. Furthermore, possible connections between chronic *H. pylori* infection and gastric carcinoma and primary gastric lymphoma are now being explored with increasing alacrity. With a few notable exceptions, pathologists have remained passive spectators of these exciting discoveries and have allowed gastroenterologists and microbiologists to set the pace in the quest for the determinants of gastritis, peptic ulcer and gastric cancer. This article is intended to outline some of the accepted facts on the development, progression, and pathology of *H. pylori* gastritis and to pose questions about this elusive infection. The authors hope that it might also contribute to stimulate further research, particularly on those aspects that are eminently suited to be addressed by pathologists.

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What is *Helicobacter pylori*?

Helicobacter pylori, known as *Campylobacter pyloridis* until indignant latinists intervened and renamed it *Campylobacter pylori*, has acquired its current (and likely permanent) name in 1989 [40], only 6 years after its first description as "unidentified curved bacilli on gastric epithelium" by Marshall [62] and Warren [78]. *H. pylori* is a Gram-negative bacterium with a distinctive spiral shape which is easily identified in histological specimens. The bacterium measures between 2.5 and 4.0 μm in length and between 0.5 and 1.0 μm in diameter; its extremities are rounded and it has two or more unipolar flagella. One of the most distinctive biochemical features of *H. pylori* is its ability to produce large amounts of urease, an activity to which it devotes a tremendous amount of its metabolic resources. Urease may have a number of important functions, including enabling the initial colonization of the gastric mucosa and allowing its survival in an acid environment [20, 49]. This property of *H. pylori* has been exploited for the development of diagnostic tests, known collectively as the urea breath tests, based on the detection of urea in the breath of infected subjects [46].

Gastritis, ulcer and cancer: is *H. pylori* the primum movens?

Chronic active gastritis (CAG) is the constellation of inflammatory responses mounted by the gastric mucosa against *H. pylori*. In the majority of infected persons throughout the world CAG is the only expression of *H. pylori* infection. However, a proportion of infected subjects develop duodenal ulcer, others atrophic gastritis with or without gastric ulcer. An even smaller proportion of the infected population develops gastric carcinoma or primary lymphoma. If we consider atrophic gastritis, peptic ulcer disease, gastric carcinoma and primary gastric lymphoma as possible outcomes of *H. pylori* infection, it becomes clear that the key to understand the

pathogenesis of these diverse conditions lies in elucidating the course of *H. pylori* infection and the host-parasite relationship in the years that precede the development of one of these outcomes.

In a fashion similar to other bacterial infections, one of two possible occurrences follows the entrance of *H. pylori* into the host: the organism may be either eliminated (because of insufficient size of the inoculum, low virulence, or effective host responses) or may initiate an infection. When it does so, in contrast to other known bacteria *H. pylori* induces an inflammatory reaction that appears simultaneously acute (polymorphonuclear response) and chronic (lymphoplasma-cellular infiltrates and development of lymphoid follicles). Although often intense and virtually always associated with local and systemic immune responses, this combined reaction appears ineffective in eradicating the organism. An apparent truce develops between host and bacterium, which allows *H. pylori* to attain a certain biomass and survive, presumably unharmed, as long as appropriate conditions exist in the gastric ecosystem. Why then, do peptic ulcer or malignancies develop in some patients?

Where is *Helicobacter pylori*?

H. pylori is where gastritis is. Previous demographic and nosologic associations with gastritis have now been documented to apply to *H. pylori* [42]. These include associations with age, low socioeconomic status, gastric ulcer, duodenal ulcer, and gastric cancer. In developing countries, *H. pylori* infection is almost universal at an early age. In these populations atrophic gastritis is common in young adults, and both gastric ulcer and gastric carcinoma are frequent, whereas duodenal ulcer disease is reportedly rare. Therefore, it is likely that the acquisition of *H. pylori* infection in early childhood results in atrophic gastritis and gastric atrophy, limiting the stomach's ability to make acid and preventing duodenal ulcer disease from appearing. Recent data from Peru support this concept, showing that in an area where childhood *H. pylori* infection was virtually universal, gastric ulcer and gastric cancer were common but duodenal ulcer was rare [5]. This hypothesis is also consistent with the previous observations concerning the epidemiology of gastric cancer in Japanese who immigrated from Japan to Hawaii [51]. Immigrants (infected with *H. pylori* in early childhood) retained the high risk they carried with them, but their children born in Hawaii, infected later in life, experienced a marked reduction in the risk of developing gastric carcinoma. The mechanisms through which *H. pylori* may promote the development of gastric carcinoma remain unknown, but *H. pylori* is believed to create the proper environment (e.g., chronic gastritis and intestinal metaplasia) on which other factors may express their carcinogenic potential [71]. The current postulate is that atrophic gastritis in tandem with one or more yet unidentified cofactors (candidates include nitrates and salt) are responsible for the observed frequency of gastric cancer in a population [8, 10, 69]. These data are consistent worldwide. In several populations there is a strong association between the prevalence of *H. pylori* prevalence and the incidence of the intestinal type of gastric carcinoma [11, 21, 25, 27, 65, 66]; furthermore, gastric carcinoma is rare in populations in which the prevalence of *H. pylori* is low. The progression from atrophic gastritis to gastric cancer may take several decades: any delay in the average age of acquisition of *H. pylori* will postpone the development of gastric cancer in that population. It will also reduce its frequency by allowing the infected individuals to reach their natural life span before sufficient time has elapsed for gastric cancer to develop. In agreement with this hypothesis, gastric cancer in the United States is most prevalent in populations of low socioeconomic status, such as blacks and Hispanics in whom *H. pylori* is acquired at an early age [12, 15, 48, 61]. Recently, four separate case control studies have provided convincing evidence that *H. pylori* infection represents an independent risk indicator of gastric cancer [26, 52, 65, 66]. Studies from Finland have shown that a very high relative risk for gastric cancer is related to gastric morphology, with the highest risk being experienced by those with severe pangastritis ("multifocal atrophic gastritis" in Correa's terminology) [9] which is considered the end result of long-standing gastritis [72, 74].

Recent studies show an even more dramatic association between *H. pylori* infection and primary gastric lymphoma of the MALT (mucosa-associated lymphoid tissue) type [19, 79]. These lymphomas do not seem to arise in tissues devoid of MALT [58]. Thus, the development of MALT appears to be a necessary first step in the genesis of these lymphomas. The formation of MALT in the stomach is a virtually universal response to *H. pylori* infection [35, 77, 81]. In addition to strong evidence from retrospective studies supporting this association, a recent report that MALT-lymphomas regressed in five patients when their *H. pylori* infection was eradicated [80] and an experimental study demonstrating that low-grade B-cell lymphomas respond to *H. pylori*-specific T-cells and their products [57] further support the concept that *H. pylori* may play a pivotal role in the genesis of these tumours.

How does *H. pylori* cause gastritis?

The steps and mediators in *H. pylori* infection and inflammation are unknown and there is no information as to what proportion of *H. pylori* ingestions result in initial colonization, nor which proportion of early infection are eradicated by the host. The difficulty in transmitting the infection to two volunteers [63, 64] who ingested bacterial cultures suggests that either infection follows ingestion only infrequently, that the host can eliminate the bacteria, or that strains cultured in vitro lack important virulence factors.

The initial steps are likely ingestion, followed by penetration of the mucus layer, "swimming" through the mucus layer to the mucosa leading to attachment and multiplication. The first host reaction is neutrophilic.

This is an important phase of the infection and one in which the bacterium and any bacterial toxins are unfettered by a local secretory IgA response. The mediators of the neutrophilic reaction at this stage are unknown, but local or systemic antibody is unlikely to be involved because the reaction precedes their appearance. One feature that was believed to be especially common during the acute phase was for *H. pylori* to "invade" between mucocytes [44]. However, studies with a new stain that allows the simultaneous visualization of *H. pylori* and gastric morphology [37] indicate that this feature is common also during the chronic phase of the infection. Bacterial products such as endotoxins, peptidoglycans and formylpeptides have been found in damaged tissue along with host products such as activated complement components. This process results in a vigorous local and systemic immune response.

The second phase of the infection includes continuation of neutrophilic inflammation but also infiltration with lymphocytes and plasma cells. The role of the local and systemic immune response in both controlling the infection and neutralizing any *H. pylori* toxins is an area of considerable interest. There is little or no information concerning the critical factors responsible for either the neutrophilic or mononuclear inflammatory response. Among the factors that deserve study are the relationship between the histological findings and bacterial density, a complicated issue from the methodological viewpoint [28]. Examination of histological sections from patients infected with *H. pylori* has yielded controversial results: several studies suggest that bacterial density may be directly correlated with the intensity of the inflammatory response [1, 4]. However, our observations indicate that, while a statistically significant relationship exists between density of *H. pylori* and intensity of the inflammatory infiltrate, the predictive value of this relationship is very low and may lack biological significance [30].

Different manifestations of *H. pylori* infection may be related to factors that reside with the host, with the organism, or both. For example, it has been hypothesized that *H. pylori* gastritis may be confined to the antrum of patients with duodenal ulcer (antral predominant gastritis) because the pre-existing high rate of acid secretion prevents effective colonization of the gastric body [41]. Several studies suggest that the pattern of gastritis in duodenal ulcer patients is different from that of other infected subjects [3, 4, 24]. In a recent analysis of three groups of infected patients with duodenal ulcer, we found distinctly distinct patterns of inflammation in patients with gastric ulcer, duodenal ulcer, and gastritis without ulcer. Duodenal ulcer patients had a greater gastritis gradient between the antrum (where organisms are more numerous and all inflammatory indexes are higher) and the corpus, supporting the hypothesis of antral predominant gastritis. Patients with gastric ulcer and non-ulcer infected subjects had an almost negligible antral-corpal gradient. Interestingly, the highest overall degrees of bacterial density and active gastritis were found in volunteers without ulcer disease [34, 36]. In addition to these indirect mechanisms, *H. pylori* may also infect areas of gastric metaplasia in the duodenal bulb [82]; the resulting inflammation and epithelial damage is believed by some authors to be one of the essential steps of duodenal ulcerogenesis [6, 17, 82].

Differences in virulence of the *H. pylori* strain may also be responsibel for the different expressions of the infection. Approximately 50 to 60% of *H. pylori* isolates produce a vacuolating toxin in vitro (Tox^+ strain), and several studies have shown that subjects infected with Tox^+ strains have increased antral polymorphonuclear inflammation when compared to subjects infected with Tox^- strains [13, 22]. These different strains of *H. pylori* may cause different degrees of inflammation through direct toxic effects on the mucosal cells or indirectly through immune mediators [14]. *H. pylori* infection is associated with a brisk humoral immune response directed against a large number of *H. pylori* proteins, both locally and systemically. One approach to identify specific strains or markers for virulence is to examine the target of IgA produced by antral mucosal biopsies and gastric mononuclear cell cultures. Along these lines of inquiry, the study of Crabtree et al. [14] provided the first clear indication of an association between a protein (or class of proteins) and the severity of the gastritis. One *H. pylori* protein, molecular weight 120000 (120K), was associated with more severe gastritis (neutrophilic infiltration and epithelial surface degeneration) and with peptic ulcer disease suggesting that (a) there are strain differences between *H. pylori*, (b) that the 120K protein is either involved in the process, or (c) is a marker for a *H. pylori* factor or *H. pylori*-host interaction leading to more severe gastritis. This is a promising area of inquiry, and further studies are needed to clarify the role of these proteins and their association with the various clinical manifestations of *H. pylori* infection.

Genetic studies using DNA-DNA hybridization in solution had previously shown that *H. pylori* obtained from volunteers with asymptomatic gastritis were in a different hybridization group that strains from patients with duodenal ulcer [83]. Preliminary data from PCR amplification of repetitive DNA elements in *H. pylori* (rep-PCR) suggest that isolates from duodenal ulcer patients form a separate cluster from those with asymptomatic gastritis [39].

Is *H. pylori* gastritis forever?

The typical appearance of *H. pylori* chronic antral gastritis (depicted in Fig. 1) has been described in detail in several histopathological studies [16, 23, 29, 56, 68, 82]. The architecture of the mucosa is well-preserved, with often only minor disarray of the pits caused by the lympho-plasmacytic infiltrate occupying the lamina propria. Neutrophils and variable numbers of eosinophils are mixed with the mononuclear infiltrate, but they are most prominent within the foveolar epithelium, where they are seen individually or in tiny clusters. Small aggregates of neutrophils, usually without fibrin, can often be seen on the surface epithelium and within the pits (pit

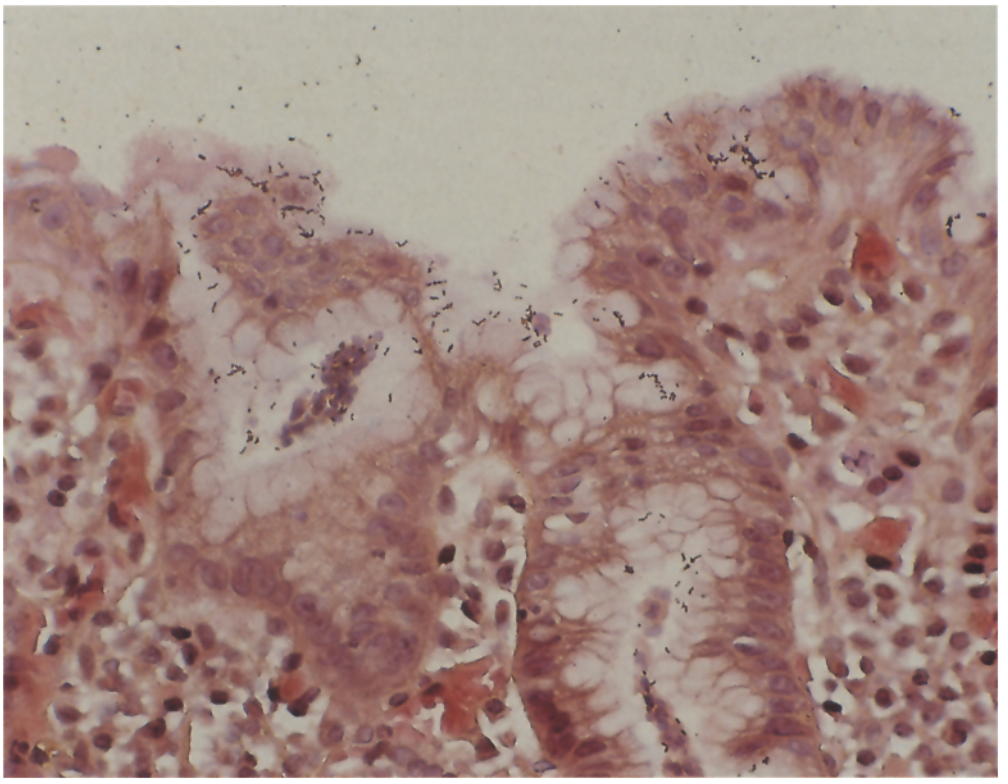

Fig. 1 Typical appearance of the antral mucosa infected with *Helicobacter pylori*. Innumerable bacteria line the epithelial surface, which shows mucin depletion and cellular disarray. Rare neutrophils infiltrate the surface epithelium and a mixed inflammatory infiltrate expands the lamina propria. Genta stain, original magnification ×400

abscesses). The antral mucous glands may be separated by the infiltrate in the lamina propria, but only exceptionally do they contain inflammatory cells within their epithelium and virtually never in the lumen. *H. pylori* organisms are located singly or in clusters along the surface and foveolar epithelium. They may appear to be entrapped within mucous strands, freely floating in the foveolar spaces, adherent to the columnar cells, or inserted between mucous cells. These apparently different locations more likely reflect the limitations of the bi-dimensional observation of a tri-dimensional structure than the real distribution of the bacteria. Only exceptionally (perhaps once in 20 or 30 infected biopsy specimens examined) are rare isolated organisms seen in the lumen of mucous glands or within the lumen of oxyntic glands. The typical appearance can vary considerably depending on the size and relative proportions of the different components of the inflammatory infiltrate. Intra-epithelial neutrophils may be so rare that they are difficult to find, even in the presence of large numbers of organisms. However, gross destruction of heavily infiltrated and dilated pits may be seen, and large numbers of neutrophils may be mixed with the background mononuclear infiltrate. Eosinophils may be prominent in some patients, for unknown reasons.

The variation in numbers of polymorphonuclear cells may be pathogenetically important, but it is rarely a source of confusion in the interpretation of gastric biopsies, and the aphorism "if you see polys look for *H. pylori*" is, or should be by now, well-embedded in every pathologist's diagnostic algorithm. Irrespective of the relative prevalence of different inflammatory cell types, one feature that, in our experience, is virtually never missing from an *H. pylori*-infected antrum is the presence of lymphoid follicles. Long regarded as normal structures of the gastric mucosa (in the days when some degree of chronic gastritis was considered by many to be part of the normal aging process), lymphoid follicles are being increasingly recognized as typical, if not pathognomonic, of *H. pylori* infection, in both adults [77, 81] and children [70]. By examining at least 8 biopsy specimens from each of 60 patients with documented *H. pylori* gastritis, we found lymphoid follicles with germinal centres in all of them, whereas none of 20 stomachs from serologically and histologically negative normal subjects contained these structures [35]. We do not know yet whether lymphoid follicles represent the expression of a specific immune response to the organisms, but studies are underway to address this issue. Occasionally, the presence of an unusually dense mononuclear infiltrate, particularly if neutrophils are few and inconspicuous, may suggest an infiltrative, or even a proliferative process such as lymphoma. In such cases compact aggregates of mononuclear cells expand the lamina propria and obfuscate the foveolar and glandular structures, giving the impression of atrophy. Furthermore, lymphocytes infiltrate into the epithelium and form lympho-epithelial lesions, a feature frequently associated with mucosa-associated lymphoid tissue lymphomas (or MALTomas) [58]. When closely observed, however, the lymphocytes have a mature, benign appearance and, in their intra-epithelial location, are surrounded by a clear halo, considere to be a hallmark of benign inflammatory infiltrates [84]. In these cases, a careful search for *H. pylori* is necessary because they tend to be rare. One must

also bear in mind that finding *H. pylori* organisms does not exclude the possibility of a concurrent lymphoma; therefore, a close follow-up of suspicious cases with extensive biopsy sampling after attempting treatment of the infection is mandatory [31].

A lymphocytic infiltrate involving the surface epithelium may be the most conspicuous response to *H. pylori* in some subjects. The resulting picture is what has been called lymphocytic gastritis, a recently described entity found to be associated with *H. pylori* infection in approximately half of the cases [18, 53].

The density of organisms in the corpus of *H. pylori*-infected stomachs is generally slightly lower than that of the antrum, but the prevalence of infection is essentially the same. In the corpus, however, there may be little or no inflammatory response along with the mucosal colonization. When inflammation is present, it often has the appearance of what used to be known as superficial gastritis [9]. A band-like infiltrate of mononuclear cells separates the slightly elongated pits from the subjacent oxyntic mucosa, and usually very small numbers of neutrophils populate the columnar epithelium. Eosinophils are frequently seen between the glandular spaces of the oxyntic mucosa, and bacteria may rarely be present in the lumen of oxyntic glands and even in the canaliculi of individual oxyntic cells (Fig. 2). Lymphoid follicles, although less prevalent than in the antrum, are often found in the corporal mucosa, even if this area shows no other evidence of infection. Although the overall inflammatory response is usually less intense in the corpus than in the antrum. Chronic active gastritis limited to the corpus (corpus predominant) does exist. There are data suggest-

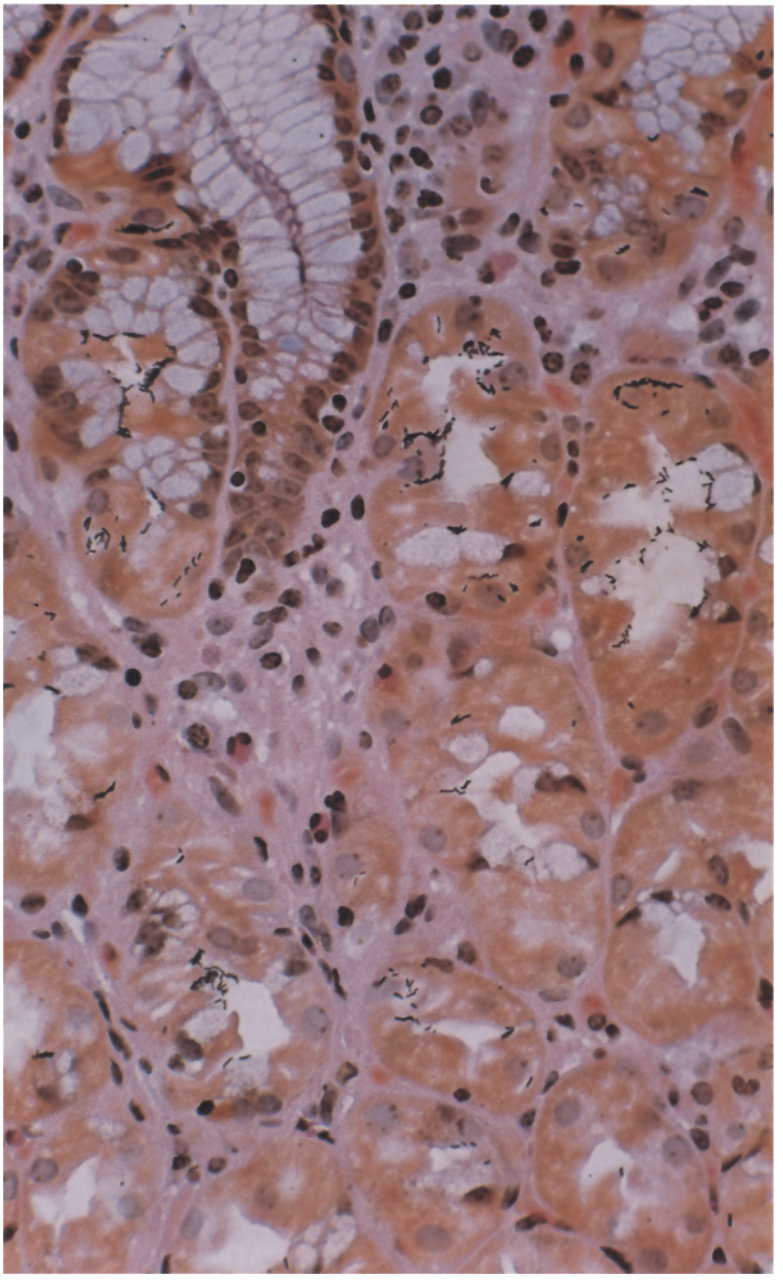

Fig. 2 An unusual case of *Helicobacter pylori* infection. Bacteria are predominantly located in the lumina of the oxyntic glands and some may be seen within the canaliculi. As it often seen in the corpus, the inflammatory response is inconspicuous. Genta stain, original magnification ×200

ing that treatment with acid pump inhibitors may reduce considerably both the bacterial density and the inflammatory responses in the antrum, while causing a proximal migration of *H. pylori* towards the corpus [76]. The existence of this drug-induced corpus predominant gastritis must be kept in mind when obtaining gastric biopsy specimens for the histopathological demonstration of *H. pylori*: the lack of adequate sampling from the corpus may result in a lower diagnostic yield.

Surface epithelial damage in various degrees of intensity almost invariably accompanies *H. pylori* infection and may occur in both antrum and corpus. These cellular alterations, described in elegant detail by Chan et al. [7] and Hui et al. [56] consist of flattening of the surface cells, formation of hyperplastic-looking cellular tufts and other subtler changes. They are found even in areas not otherwise damaged by intra-epithelial inflammatory cells and are believed to represent the direct effects of the adhering organisms [55]. Some degree of mucus depletion, often so pronounced to give the cells a cuboidal appearance, is found in the gastric mucosal epithelium of virtually all infected subjects, irrespective of whether bacteria are seen at in that particular location.

Our understanding of the natural history of the histopathological features of *H. pylori* gastritis is limited by the dearth of prospective long term cohort studies. With the exception of an extraordinary number of sequential endoscopic and pathological studies conducted in Finland and Estonia over a period of more than 30 years by a farsighted team led by Max Siurala (a gastroenterologist) and Pentti Sipponen (a pathologist), and Correa's meticulous work of in Colombia, the natural evolution of gastritis has not been directly investigated. Data obtained from these authors' work as well as from cross-sectional studies in populations with high prevalence of *H. pylori* infection suggest that in a considerable percentage of patients the inflammation may eventually become exclusively mononuclear and slowly vanish over a period of years, while at the same time the gastric glands decrease in number, the surface and foveolar epithelium undergoes intestinal metaplasia. The sequence of these changes represents the hypothetical pathway from chronic active gastritis to atrophic gastritis and eventually to atrophy and intestinal metaplasia. This latter change is believed to lay the foundations on which dysplasia and carcinoma develop [10, 12, 71, 73, 74].

In search of therapy

In light of the unequivocal evidence linking *H. pylori* infection with peptic ulcer and the increasing body of indirect information suggesting a correlation with cancer and lymphoma, the concept that ulcer patients with *H. pylori* infection must be treated is gaining acceptance [44] and has been sanctioned by a recent consensus conference held at the National Institutes of Health, Bethesda, Maryland, in February 1994. At the present time, treatment of asymptomatic infected subjects is not generally recommeded. Nevertheless, numerous studies are underway to determine if any subgroups among these asymptomatic patients are more likely to develop clinical disease and, therefore, should be treated before that occurs. Successful therapy can be achieved with a variety of combinations of antibiotics, bismuth and, more recently acid pump inhibitors [50, 54], usually named after the number of the ingredients of the cocktail (monotherapy triple therapy, etc.). Irrespective of the methods used to achieve it, cure of *H. pylori* infection results in the rapid disappearance of neutrophils from the gastric mucosa, which can be seen as early as two or three days after the beginning of triple therapy [38]. Long term studies of treated patients are rare. We have studied 15 subjects with histologically documented *H. pylori* infection for periods of 2 to 3 years post-therapy by repeated endoscopies and extensive biopsy sampling. The rapid disappearance of neutrophils was accompanied in most subjects by an initial increase in eosinophils, while the degree of mononuclear inflammation decreased at a much slower pace, in some cases taking more than 1 year to return to normal. Lymphoid follicles with germinal centres did not completely disappear in any of the subjects within the time of the study [33].

A practical note

If, at the time of endoscopy, it is important to confirm whether *H. pylori* infection is present, one should use techniques that result in a permanent record and provide objective data concerning the presence of the bacteria and the status of the gastric mucosa. The simplest approach is histological examination of mucosal biopsy specimens. Best results are obtained when large biopsies (at least two, as outlined above) are obtained from the antrum. To determine what are the best sites to biopsy for the histopathological diagnosis of *H. pylori*, we examined the complete gastric mapping biopsies from 89 patients with known *H. pylori* infection [32]. The density of *H. pylori*, neutrophils and lymphoid follicles was assessed semi-quantitatively in each biopsy site. The likelihood of getting a false-negative biopsy was also calculated for each site. All biopsies from the antral lesser curvature, at or near the incisura, had detectable *H. pylori*. This area as well as the cardia had higher scores than the gastric corpus, but most differences were not significant. Less than 3% of antral biopsies were false negative compared to between 6% and 9% in the corpus. Neutrophils were present in >94% of all antral biopsies compared to 60% to 86% from the corpus. Lymphoid follicles were detected in approximately two-thirds of antral biopsies compared to less than half of those from the corpus. We concluded that two antral biopsies (one from the lesser and one from the greater curvature) will yield virtually 100% sensitivity for detecting *H. pylori* infection. Biopsies from the corpus should be obtained for the evaluation of distribution and severity of gastritis but will not increase diagnostic yield unless there is extensive intestinal metaplasia in the antrum or the patient has received acid pump inhibitors therapy. The presence

of neutrophils and lymphoid follicles have a high positive predictive value but their absence, particularly in the corpus, dos not exclude *H. pylori* infection.

A number of factors must be considered for optimal specimen evaluation [2]. Since *H. pylori* predominantly inhabits the mucous layer overlying the stomach and within the gastric pits, biopsies should be handled in such a manner so as to minimize tissue distortion and to dislodge as little mucus as possible. Thus, methods that involve placing the fresh biopsy on a supporting structure such as a piece of filter paper should be avoided. Although many of the biopsies tend to curl due to contraction of the smooth muscle of the muscularis mucosae, optimal orientation of the tissue at the time of paraffin embedding is easily achieved by a trained technician. A number of methods for identification of bacteria in tissue sections have been suggested including the modified Giemsa, the Gimenez stain, and a number of Gram stains, (Brown-Brenn, Brown-Hopps, and half Gram). Each of these vary in difficulty, cost, ease of interpretation, and differential staining of the tissue. Recently, by combining three commonly available stains (Steiner, haematoxylin and eosine and alcian blue at pH 2.5) into a single procedure we have developed a stain that permits the optimal detection of *H. pylori* in tissue sections, while simultaneously allowing the histopathological evaluation of all salient characteristics of the gastric mucosa [37]. This procedure is inexpensive, as easy to perform as any silver stain, and provides consistent results. This stain is as sensitive as the Warthin-Starry for the detection of *H. pylori* and significantly more sensitive than haemtoxylin and eosine alone (>99% vs. 85% when compared to the Warthin-Starry in a series of 332 positive biopsy specimens; >99% vs. 61% in 49 biopsy specimens with rare bacteria). This stain is particularly useful for the visualization of small numbers of bacteria, such as in the evaluation of post-treatment gastric biopsies, in specimens with abundant mucus or debris, and in specimens from the corpus, where *H. pylori* does not usually elicit strong inflammatory responses and, therefore, pathologists' index of suspicion tends to be low.

Culture

Culture of *H. pylori* is generally unnecessary and most hospital laboratories have a relatively low yield of positive cultures, even in patients known to be infected. Furthermore, culture can easily add several hundred dollars to the cost of the procedure. If antibiotic sensitivity testing becomes useful to guide therapy, culture may become important; it is not now. With present knowledge and therapies, culture is not recommended, except in research settings. If the specimens are kept at 4° C, processing of tissue specimens for culture can be safely delayed more than 4 h without an appreciable decrease in growth. A transport medium is recommended, especially if the microbiology laboratory is located at some distance from the endoscopy unit and a delay in transportation or processing is expected. Possible transport media include Cysteine Brucella broth, normal saline, and glucose. In our experience, Cysteine Brucella broth with 20% glycerol is a good choice because it is an excellent transport medium and biopsy specimens can also be frozen in it (at −70° C) without loss of *H. pylori* viability for more than a year. *H. pylori* is intimately associated with the gastric mucosa, and culture from tissue biopsies has a yield superior to that from gastric aspirates or brushings [60, 75].

Where do we go from here?

Although the basic morphological features of *H. pylori* gastritis may have been described in sweeping detail, the histopathologist's contribution is far from exhausted. The nature of the relationship between *H. pylori* and type and intensity of inflammation of the mucosal responses remains an unsettled issue. Although it is clear that patients with duodenal and gastric ulcer have a different distribution of gastritis, the topography of *H. pylori*-associated gastritis in the years before the development of peptic ulcer is not known. Anecdotal evidence from various parts of the world suggests that in developing countries and underprivileged populations not only is the prevalence of *H. pylori* infection greater than in more affluent areas and groups, but also the magnitude of the infection (assessed by the numbers of bacteria seen on the gastric mucosa) is substantially greater in individual patients from the poorer strata of the society. If confirmed, this finding would indicate that reinfection is of crucial importance in maintaining the levels of the gastric bacterial biomass and, perhaps, might be a factor in promoting gastric cancer. These and other similar issues can only be resolved by the painstaking microscopic examination of carefully collected and skillfully processed and stained gastric biopsy specimens. Pathologists willing to devote their time and expertise to the solution of these problems will make a substantial contribution to our understanding of the pathogenesis of the protean conditons asscіated with *H. pylori* infection of the gastric mucosa.

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