Histologic Characteristics of *Campylobacter pylori* (Helicobacter pylori) Mediated Gastritis*

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ABSTRACT

One hundred-nineteen specimens were reviewed to determine whether or not there were histologic changes specific for *Campylobacter pylori* (CP), (*Helicobacter pylori*) mediated gastritis.

Hematoxylin and eosin (H&E), Brown-Hopp, and Wright-Giemsa stained sections were examined independently by two pathologists for (a) the presence of acute cryptitis, (b) percent and degree of crypt involvement, and (c) spectrum of inflammatory cells within the lamina propriae. The amount of mucus was quantified on the Periodic Acid Schiff (PAS)-Alcian Blue stain sections. Changes in the character of the mucus were noted by using both the PAS-Alcian Blue and the High Iron Diamine-Alcian Blue. A positive specimen for *Campylobacter pylori* (CP +), (*Helicobacter pylori*) was defined as one in which curved or spiral shaped microbes were identified on Wright-Giemsa and Brown-Hopp stain. Seventy-eight specimens were CP + and 41 CP −. Statistically significant histologic findings included the extent and degree of superficial cryptitis and the preponderance of plasma cells in CP + cases. These findings confirm aspects seen in an animal model and suggest that there is an histologic pattern consistent with *C. pylori* (*Helicobacter pylori*) mediated gastritis.

Introduction

*Campylobacter pylori* (*Helicobacter pylori*) was first described as spiral-shaped microbes in the stomachs of animals in 1893 and in human stomachs in 1906; however, they were largely ignored until 1983 when Warren and Marshall began to report similar curved-shaped bacilli in the antral gastric mucosa of patients with chronic, active gastritis and gastro-duodenal...
ulcers. Over the intervening years, others have confirmed these observations, but controversy still exists as to the pathogenicity of \( \text{C. pylori} \). If these microbes do contribute to the development of non-ulcer dyspepsia and peptic ulcer disease, the inflammatory response of the gastric mucosa may be qualitatively or quantitatively different when these bacteria are present. Krakowka et al., for example, have shown that \( \text{C. pylori (Helicobacter pylori)} \) infection of the antral mucosa in the neonatal gnotobiotic pig was associated with a depletion of mucus production. If this also occurs in humans, the pathogenicity of \( \text{C. pylori (Helicobacter pylori)} \) is certainly implied.

In this study, human antral biopsy specimens were separated into those containing \( \text{C. pylori (Helicobacter pylori)} \) from those that did not. It was then determined whether or not there was any difference in either the mucosal inflammatory response or mucus content/character in the two groups. The human data reveal that the presence of \( \text{C. pylori (Helicobacter pylori)} \) is commonly associated with acute active gastritis, and that there are subtle changes in the antral mucosa brought about by these organisms.

**Materials and Methods**

**Patients**

Patients selected for study had at least a two week history of upper gastrointestinal symptoms. The differential diagnosis prior to endoscopy was non-ulcer dyspepsia versus peptic ulcer. No patient was included with any clinical evidence of peptic ulcer disease at the time of endoscopy.

**Specimen Preparation**

All antral biopsy specimens were placed in a 10 percent buffered formalin solution until processed in a Miles VP 1000 Tissue Processor.* These specimens were sectioned and heated at 75°C for 24 hours. Four stains were used—(1) hematoxylin-eosin (H&E),† (2) Giemsa,‡ (3) Brown-Hopps, and (4) PAS-Alcian Blue and High Iron Diamine-Alcian Blue.  The PAS-Alcian Blue staining procedure consisted of bringing the section to water, followed by immersing the slide in three percent glacial acetic acid for 30 minutes and then in Alcian Blue for another 30 minutes. The slide was then rinsed in distilled water and placed in Schiff's solution for 10 minutes. It was then treated with a sodium metabisulfite.

The High Iron Diamine-Alcian Blue staining procedure began by oxidizing each deparaffinized section for 10 minutes with one percent of \( \text{H}_5\text{I}_5 \) followed by a five-minute rinse in running tap water. The section was then immersed in a solution containing 120 mg N,N-dimethyl-m-phenylene diamine (HCl), 20 mg N,N-dimethyl-p-phenylene diamine (HCl), 1.4 ml neutral formalin, 10 percent ferric chloride, and 50 ml distilled water.

The Giemsa stain was used to identify \( \text{C. pylori (Helicobacter pylori)} \) as previously described by Marshall (figure 1). The Brown-Hopps stain was employed to confirm the presence of this microbe.  

**Histologic Evaluation and Data Analysis**

Hematoxylin and eosin, Brown-Hopps, and Giemsa stained sections were examined independently by two pathologists without any knowledge of patient origin. In addition, neither

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* Miles Laboratories, Inc., Naperville, IL.
† Fisher Diagnostic, Orangeburg, NY.
‡ GAM RAM, Garden Grove, CA.
§ Polyscientific, Bayshore, NY.
‖ Sigma, St. Louis, MO.
observer knew which H&E section corresponded to the Giemsa and Brown-Hopps sections.

The inflammatory response was characterized by the extent and degree of crypt involvement. An affected crypt was defined as one in which polymorphonuclear (PMN) leukocytes had invaded the gastric epithelial cells. The extent affected was expressed as a percent of the crypts with PMN infiltrate. The degree affected was defined as the average number of PMNs per affected crypt.

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\text{Extent Affected} = \frac{\text{number of affected crypts}}{\text{total number of crypts}} \times 100
\]

\[
\text{Degree Affected} = \frac{\text{total number of PMNs}}{\text{number of affected crypts}}
\]

A crypt abscess was defined as a collection of PMNs surrounded by crypt epithelium. The number of PMNs in such abscesses was not determined.

Three hundred inflammatory cells were counted within the lamina propriae in order to characterize the composition of the infiltrate. Cell counts were not performed in either lymphoid aggregates or lymphoid follicles.

Mucus depletion was identified by a reduction in number of mucus granules in the mucus producing surface cells on the antral mucosa. The reduction in granules had to equal or exceed two-thirds normal content before the specimen was considered to demonstrate mucus depletion. Variations in mucus composition were determined with the PAS-Alcian Blue stain which turn acid mucopolysaccharides blue and neutral mucopolysaccharides red to purple. This High Iron Diamine-Alcian Blue stain will turn sialomucin blue and sulfomucin black.9,20

All histologic data was organized on a standard form and analyzed by a one-way ANOVA* using a statistical package available in Sigma Plot.† Significance was considered a p value less than or equal to 0.05.

Results

Antral biopsies were obtained from 119 male patients ranging in age from 37 to 63 years (average 45 years). The typical specimen consisted of more than one fragment (range 1 to 4). The average fragment measured 0.2 cm × 0.2 cm and contained an average of 23 crypts (range 5 to 100).

* Used as a statistical analysis.
† Jandel Scientific, Corte Madre, CA.
Of the 119 biopsies examined, 78 contained \( \text{C. pylori} \) (CP+) (\textit{Helicobacter pylori}) and 41 did not (CP-). The Giemsa and Brown-Hopps stains were equally effective in identifying this microorganism, and the results were always consistent. Both CP+ and CP— specimens manifested some degree of acute inflammatory infiltration, but the extent and degree of crypt involvement was greater in the CP+ samples (table I). On the average, the CP+ specimens had 19 affected crypts (extent affected) as compared to only seven in the CP— group (p < 0.001). Likewise, affected crypts in the CP+ samples contained an average seven PMNs (degree affected) compared to three PMNs/crypt in the CP— specimens (p < 0.01). Cryptic abscesses were present in 15 of 78 CP+ versus eight of 41 CP— samples, but this difference was not statistically significant.

\( \text{C. pylori} \) (\textit{Helicobacter pylori}) infection of the antral mucosa was associated with a reduction in the relative amount of lymphocytes in the lamina propriae (table I). The mean lymphocyte count was 41 percent in the CP— specimens as compared to 26 percent in the CP+ group (p < 0.001). Conversely, lymphoid aggregates/follicles were far more common in the infected specimens (26/78 CP+ versus 6/41 CP—, p < 0.01). The relative number of plasma cells was higher in the lamina propriae of the CP+ group (46 percent versus 38 percent in CP—, p < 0.05), but there was no significant difference in the relative numbers of immunoblast or eosinophiles in the CP+ and CP— specimens.

While all 119 antral biopsies were stained with PAS-Alcian Blue and High-Iron Diamine-Alcian Blue, only 77 showed enough staining activity for evaluation in both preparations. Of these 77, only 55 contained \( \text{C. pylori} \) (\textit{Helicobacter pylori}). Acute cellular infiltrate was evident in both CP+ (38/55) and CP— (14/22) specimens and the cellular composition of these two sub-groups was not different from that described for all CP+ and CP— samples.

All 77 specimens had neutral mucin granules in the superficial gastric epithelial cells. There was evidence of mucin depletion in 34 of 55 CP+ specimens, while only five of 22 of the CP— group met the criteria for mucus depletion (p < 0.001) (figures 2 and 3). Mucin depletion tended to be focal in nature and present both in areas of intestinal metaplasia and those with normal gastric architecture. The mucus depletion associated with \( \text{C. pylori} \) (\textit{Helicobacter pylori}) infection of the gastric mucosa did not affect the incidence of PMNs infiltration. Twenty-three of the 34 CP+ (67 percent) samples with mucin depletion had PMNs in the gastric crypts as compared with 15/22 (71 percent) of the CP— group. Likewise, when the 77 were divided into those with and without acute cellular infiltrates (PMNs in the gastric epithelium), there was no significant difference in the incidence of mucin depletion between the two groups. Con-
subsequently, there was no evidence of any relationship between mucin depletion and the presence of acute inflammatory cells in the gastric epithelium. The High-Iron Diamine-Alcian stain identified sulfomucin granules in the superficial mucin producing cells of 14 specimens, but they were evenly distributed between the CP+ and CP− groups.

Discussion

The results of this study clearly indicate that there are changes in the normal histology of the gastric mucosa following C. pylori (Helicobacter pylori) infection. These changes include: (1) Increases in the extent and degree of inflammation in gastric crypts; (2) A decrease in the relative number of lymphocytes in the lamina propriae; (3) An increased incidence of lymphoid aggregates/follicles in the lamina propriae; (4) An increase in the relative number of plasma cells in the lamina propriae; and (5) A reduction in mucin present in the superficial gastric mucosal cells.

The organism is exquisitely adaptive to the gastric mucosa6,21 and tends to be confined to the luminal surfaces of the
gastric mucus secreting cells. The retarded invasive properties of the organisms may be a function of the local immunologic effects of IgA. It is known that IgA facilitates entrapment of particles on the mucous coating. Antibody studies performed on gastric secretion support this concept, showing the presence of \textit{C. pylori} (\textit{Helicobacter pylori}) specific IgA's in gastric secretions.\textsuperscript{5,13,18} The results of our study suggest that there are more cells in the lamina propria of infected patients that can mediate immune products than in non-infected stomachs. Although histologic overlap does exist between CP+ and CP− cases, in cases where the organism is present, the mucosa appears to be immunologically active.

This is most apparent as illustrated by the preponderance of lymphoid follicles/aggregates and plasma cells in the CP+ cases. The incidence of lymphoid follicles/aggregates (figure 4) resembles to some extent \textit{Yersinia enterocolitica} infections of the colon. In this case, the prominence of lymphoid follicles is part of the histologic manifestations of infection. The presence of both lymphoid aggregates and follicles suggest antigenic stimulation is occurring on the gastric mucosa. Other histologic features of \textit{C. pylori} (\textit{Helicobacter pylori}) infections have analogy with other infectious, and inflammatory conditions of the bowel. The presence of polymorphonuclear leukocytes invading the epithelium and forming microabscess can be seen not only in ulcerative colitis, but in infections mediated by \textit{Campylobacter jejuni}.

This microbe produces soluble products, toxins or enzymes which could also serve as chemotoxins or as antigens. Slomiany et al\textsuperscript{19} have characterized a muco-protease produced by \textit{C. pylori} (\textit{Helicobacter pylori}). This postulated muco-depleting substance suggests that infection begets muco-depletion resulting in a weakened barrier and injury to the mucosa and that this triggers the invasion of PMNs. In looking at our human material, it appears as if the presence of the microbe is associated with muco-depletion, while the presence of acute inflammation in the gastric epithelium was not statistically associated with depletion. Krakowka, using a neonatal gnotobiotic piglet model of \textit{C. pylori} (\textit{Helicobacter pylori}) infection showed the initial response to be that of acute inflammation with epithelial destruction.\textsuperscript{7} This may be the histologic mani-

\textbf{Figure 4.} The antral biopsy demonstrates a developing lymphoid follicle with characteristic follicular center and T-zone mantle. This biopsy was demonstrated to have \textit{Campylobacter pylori} in the gastric crypts. (Hematoxylin and eosin, 10×.)
festation of a cytotoxin or the result of a muco-depleting substance. Our material did not show this phenomenon; acute cryptitis was not seen without a chronic component being present. However, our material most likely represents a later phase in the disease process. The majority of our patients had their symptoms for more than two weeks prior to endoscopy and may represent a later phase. The role of the infectious agents in mucus depletion cannot be evaluated in our patient material, although no relationship between the acute inflammation of the gastric epithelium was identified.

The type of mucus in patients with *C. pylori* (*Helicobacter pylori*) infection was no different from those without. The rate of intestinal metaplasia (as measured by the presence of sulfomucin) was no greater in CP+ verses CP− cases. This suggests that intestinal metaplasia is the result of the chronic gastritis-irrespective of causative agent. It is becoming clear that chronic gastritis and peptic ulcer disease may not be a single disease. Our data suggest that a definite histologic pattern may exist for those cases of infectious gastroenteropathy mediated by *C. pylori* (*Helicobacter pylori*), and that infection may be but a single cause for gastritis/peptic ulcer disease.

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References


