Helicobacter pylori Infection. Several studies on pathology and clinicopathology

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CHAPTER 4

*Helicobacter pylori*-associated Gastric Diseases

And Lymphoid tissue hyperplasia

in Gastric Antral Mucosa

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Abstract

AIM: To investigate the relationship between *Helicobacter pylori*-associated gastroduodenal diseases and lymphoid tissue hyperplasia in the antral mucosa and to pursue its evolution after eradication of *H. pylori*.

METHODS: Gastric antral biopsy specimens were obtained from 438 patients with *H. pylori*-positive gastroduodenal diseases (185 chronic gastritis, 69 gastric ulcer and 184 duodenal ulcer) and 50 *H. pylori*-negative healthy controls. Lymphoid follicles and aggregates were counted and other pathologic features were scored according to the updated Sydney System for Classification of chronic gastritis. After a course of anti-*H. pylori* therapy, biopsy specimens were obtained at 4-6 weeks, 12 and 24 months in the chronic gastritis patients group.

RESULTS: Total prevalence of lymphoid follicles and aggregates in the biopsies was 79.9% (350/438). The prevalence and density of lymphoid follicles and aggregates were significantly different in the various gastroduodenal diseases. The highest prevalence and density of lymphoid follicles and aggregates occurred in 89.9% and 0.82, respectively, in patients with gastric ulcer. The lowest prevalence of lymphoid follicles and aggregates was found in 74.6% in patients with chronic gastritis, and the lowest density of lymphoid follicles and aggregates was detected in 0.56 in patients with duodenal ulcer. The prevalence and density of lymphoid follicles and aggregates correlated strongly with the activity and severity of gastric antral mucosal inflammation. Eradication of *H. pylori* resulted in a decrease in the prevalence and density of lymphoid follicles and aggregates.
CONCLUSION: Prevalence and density of lymphoid follicles and aggregates in gastric antral mucosal biopsies correlated closely with \textit{H. pylori} infection.

Key Words \textit{H. pylori}-associated gastroduodenal diseases; Lymphoid follicles; Lymphoid aggregates, Eradication
Introduction

Infection with *H. pylori* is a major cause of chronic gastritis, and may lead to the formation of gastric mucosa-associated lymphoid tissue (MALT) and the occasional development of primary gastric B-cell lymphoma. The normal gastric mucosa contains very few lymphocytes in the lamina propria. Lymphoid follicles and aggregates are characteristic of *H. pylori*-associated gastritis. Some studies have shown various prevalences of lymphoid follicles, varied from 27.4% to 100%, in gastric mucosa of patients with *H. pylori*-associated gastritis.

In order to examine the relationship between *H. pylori* and the development of lymphoid tissue hyperplasia, we investigated the evolution between lymphoid follicles and aggregates and other histopathologic features during the 2-year observation period after eradication of *H. pylori*. We tried to answer the following questions: (1) Is there a difference in the overall prevalence and density of lymphoid follicles and aggregates in various gastroduodenal diseases? (2) Is there a relationship between lymphoid follicles and aggregates and the density of *H. pylori* colonization, severity and activity of inflammation, atrophy and intestinal metaplasia? (3) Do lymphoid follicles and aggregates regress in number or disappear after successful eradication of *H. pylori*.

Materials and methods

Study Subjects

A total of 438 patients with *H. pylori* infection were recruited from those visiting
the endoscopy unit at our hospital during May 1995 and August 1996. The patients, including 282 men and 158 women with ages of 18 to 72 years (mean age, 41.1 ± 10.1), were divided into three groups: (1) Group 1 consisted of 185 dyspeptic patients with chronic gastritis (105 superficial chronic gastritis and 80 atrophic gastritis proven by endoscopy and histopathology); (2) Group 2 consisted of 69 patients with endoscopically proven gastric ulcer; (3) Group 3 consisted of 184 patients with endoscopically proven duodenal ulcer. Fifty *H. pylori*-negative dyspeptic patients with endoscopically and histologically confirmed normal gastric mucosa were selected as control group.

**Endoscopy and biopsy Sampling**

All patients underwent upper gastrointestinal endoscopy before treatment. Those patients with chronic gastritis underwent repeat endoscopy after treatment. According to the mapping principle of our study, during each endoscopic examination, six antral mucosal biopsies were taken from the anterior and posterior wall, and the lesser and the greater curvature within 2~3 cm from the pylorus. Additional biopsy specimens were taken from any lesion areas when necessary. Two antral biopsy specimens were used for microbiological assessment (rapid urease test and culture), the others were used for histologic assessment.

**Histopathology**

All antral biopsy specimens for histologic examination were fixed in 10% formalin, and embedded in paraffin on oriented edge, cut in sequential 5 μm sections. All
tissue sections were stained with haematoxylin and eosin for histologic examination and with a Giemsa stain for *H. pylori* assessment.

The severity and activity of gastritis, and atrophy and intestinal metaplasia, as well as *H. pylori* density were evaluated according to the updated Sydney System\textsuperscript{5}. The infiltration of gastric mucosa by mononuclear cells and polymorphonuclear leucocytes, atrophy and intestinal metaplasia were graded as following: 0=none, 1=mild, 2=moderate, 3=severe. Atrophy was defined as the loss of inherent glandular tissue, with or without replacement by intestinal-type epithelium. For optimal histological evaluation, all gastric biopsy specimens included surface epithelium and muscular's mucosae, and small pinch biopsies were excluded from our study. A total of 1660 gastric biopsy specimens were investigated and analyzed. A lymphoid follicle was defined as an aggregate of lymphocytes with a germinal center. Lymphoid aggregates was defined as an accumulations of lymphocytes and plasma cells without a germinal center. Lymphoid tissue hyperplasia in gastric mucosa consist of lymphoid follicle and aggregates in each antral biopsy specimen were counted and tabulated. The density of lymphoid follicles and aggregates (the number of biopsy specimens with lymphoid tissue hyperplasia / the number of biopsy specimens examined) represents the intensity of lymphoid follicles and aggregates in the antral gastric mucosa.

All gastric specimens, including those from the normal control group, were investigated and scored independently by two pathologists (Xiao-Yu Chen and Yao Shi). The pathologists were blinded to any clinic information. Before starting the
study, the two pathologists reached consensus about the methods of grading the features of gastritis through interactive sessions at a multihead microscope. Divergences were resolve by consensus.

**H. pylori Assessment**

*H. pylori* assessment was performed by culture and histopathology. Patients were positive for *H. pylori* if *H. pylori* was present in culture and/or tissue sections. Eradication of *H. pylori* infection was defined as the absence of microorganisms in both culture and tissue sections 4-6 weeks after cessation of therapy.

**Treatment and follow-up study**

Eligible patients with *H. pylori* positive chronic gastritis were given a 7-day course of bismuth triple therapy. The patients again underwent endoscopy and biopsy at 4-6 weeks after completion of treatment. Complete sets of gastric antral biopsy specimens were obtained from 35 and 7, respectively, of those patients with *H. pylori* negative 12 and 24 months after successful eradication of *H. pylori*. The histopathologic changes and lymphoid tissue hyperplasia in those biopsy specimens were reinvestigated.

**Statistical analysis**

Two-tailed Chi square and Fisher exact probability were used when appropriate. Statistical significance level was set at p<0.05.
Results

The prevalence and the density of lymphoid follicles and aggregates in various gastroduodenal diseases

The prevalence of lymphoid follicles and aggregates in various gastroduodenal diseases was shown in figure 1. Overall, lymphoid follicles and aggregates were present in the antral mucosa of 350 (79.9%) patients. The prevalences of lymphoid follicles and aggregates in various gastroduodenal diseases were significantly different. The highest number of lymphoid follicles and aggregates occurred in 89.9%(62/69) in the patients with gastric ulcer. The lowest prevalence of lymphoid follicles and aggregates was found in 74.7%(138/185) in the patients with chronic gastritis. The prevalence of lymphoid follicles and aggregates between patients with chronic gastritis and gastric ulcer has significantly difference (p<0.01). The presence of lymphoid follicles and aggregates in the gastric antral mucosa was higher in the patients with gastric ulcer than those with duodenal ulcer, but did not reach statistical significance (p>0.05).
Figure 1 The prevalence of lymphoid follicles and aggregates in the antral mucosa in different groups of patients

CG: Chronic gastritis; GU: Gastric ulcer; DU: Duodenal ulcer

A total of 1476 eligible gastric antral biopsy specimens from different groups (669 chronic gastritis, 239 gastric ulcer and 568 duodenal ulcer) were analyzed, and the number of lymphoid follicles and aggregates in each gastric antral mucosa was graded from 0 to 6 (averaged 0.7). The overall density of the lymphoid follicles and aggregates was 0.71 (1031/1476) in the infected antral mucosa. The density of the lymphoid follicles and aggregates in gastric antral mucosa was also shown significantly different and paralleled with the prevalence of lymphoid follicles and aggregates in the various gastroduodenal diseases. The highest and lowest density of lymphoid follicles and aggregates occurred in 0.82 (196/239) and 0.56 (318/568), respectively, in the patients with gastric ulcer and duodenal ulcer (Fig. 2).
Statistical analysis reached significant difference in the density of lymphoid follicles and aggregates between patients with chronic gastritis and duodenal ulcer, or those with gastric ulcer and duodenal ulcer ($p<0.0001$). Although the density of lymphoid follicles and aggregates was higher in the patients with gastric ulcer than those with chronic gastritis, there was no statistical difference ($p=0.105$).

*517/669  196/239  318/568
CG  GU  DU
Biopsies

Figure 2  The density of lymphoid follicles and aggregates in the antral mucosa in different groups of patients

*: The number of biopsy specimens with lymphoid tissue hyperplasia / the number of biopsy specimens examined
There were very few scattered lymphocytes and no lymphoid tissue hyperplasia in the antral lamina propria in the normal control group.

Relationship between lymphoid follicles and aggregates and pathologic features in chronic gastritis group

The relationship between lymphoid follicles and aggregates and pathologic features in the chronic gastritis group was shown in figures 3-5. The prevalence of lymphoid follicles and aggregates was higher in the patients with atrophic gastritis (88.7%, 71/80) than those with superficial gastritis (63.8%, 67/105). The prevalence and density of lymphoid follicles and aggregates were closely correlated with the severity and activity of inflammation. With an increasing degree of chronic inflammation in the gastric antral mucosa, higher numbers and density of lymphoid follicles and aggregates were detected (Fig. 4). The number of lymphoid follicles and aggregates was significantly higher in the patients with active gastritis than in those without active gastritis (p<0.0001) (Fig. 3). The correlations between the detection of lymphoid follicles and aggregates and atrophy or and intestinal metaplasia were evaluated in figure 5, a higher prevalence was detected in atrophic and intestinal metaplasia mucosa (p<0.001). With an increasing degree of atrophy and intestinal metaplasia, however, lower prevalence and density of lymphoid follicles and aggregates were found in the gastric antral mucosa.
Figure 3 The relationship between the density of lymphoid follicles and aggregates and activity in chronic gastritis group

Figure 4 The relationship between the density of lymphoid follicles and aggregates and severity of inflammation in chronic gastritis group
Evolution of the prevalence and density of lymphoid follicles and aggregates following *H. pylori* eradication in chronic gastritis group

The evolution of the prevalence and density of lymphoid follicles and aggregates in the gastric antral mucosa before and 4~6 weeks, 12 and 24 months after treatment were shown in figures 6 and 7. Eradication of *H. pylori* infection resulted in decrease of lymphoid follicles and aggregates in gastric antral mucosa. Successful eradication of *H. pylori* has obtained in 132 out of 185 (71.4%) patients after a triple antibiotic therapy. The prevalence of lymphoid follicles and aggregates before treatment was 74.6% (138/185) and decrease to 57.3% (106/185) 4~6
weeks after treatment (p<0.001). When the number of lymphoid follicles and aggregates in the antral mucosa was analyzed, the patients without *H. pylori* infection had significantly declined (p<0.001), whereas those with *H. pylori* infection remained had decreased slightly (p=0.134) after treatment compared with those before treatment. The density of *H. pylori* colonization decreased significantly in 53 patients in whom *H. pylori* infection remained 4-6 weeks after treatment. *H. pylori* colonization was only observed in 62 of 188 biopsy specimens in those patients.

![Graph showing the evolution of prevalence of lymphoid follicles and aggregates in the antral mucosa in chronic gastritis group during the two years follow up period.](image)
The prevalence and density of lymphoid follicles and aggregates decreased to 22.9% and 0.12, respectively, in 35 follow-up patients with *H. pylori* negative one year after treatment. Further a small but steady declined in the frequency and density of lymphoid follicles and aggregates (14.3% and 0.11, respectively) was observed in the antral mucosa in 7 follow-up patients with *H. pylori* negative 2-year after treatment.

*= The number of biopsy specimens with lymphoid tissue hyperplasia / the number of biopsy specimens examined.*

![Figure 7](image_url)

**Figure 7** The evolution of the density of lymphoid follicles and aggregates in the antral mucosa in chronic gastritis group during the two years follow up period
Discussion

Wyatt and Rathbone\textsuperscript{11} investigated 419 pairs of antral and corporal mucosal biopsy specimens and found lymphoid follicles in 27.4\% of patients with \textit{H. pylori}-associated gastritis. Eidt and Stolte\textsuperscript{8} studied "two to three antral specimens" from 2692 patients with \textit{H. pylori} infection and detected lymphoid follicles and aggregates in 54\% of those patients. Genta and Hammer\textsuperscript{9} found the prevalence of lymphoid follicles was in 63.8\% (110/174) of patients with chronic gastritis and in all patients with \textit{H. pylori} infection. In our study, the presence of lymphoid follicles and aggregates in the gastric antral mucosa was found in 76.0\% (350/438) of patients with various \textit{H. pylori}-associated gastric diseases. The differences in the prevalence of lymphoid follicles and aggregates in the gastric mucosa between other previously published data and our study might be due to several factors: (1) Biopsy sites may be different in the various studies. It has been known for some time that the frequency of lymphoid follicles and aggregates is obviously lower in oxyntic mucosa (14.8\% to 44.0\%) than that in antral mucosa\textsuperscript{8,10}. (2) The number of biopsy specimens taken varied in those studies. Most data about lower prevalence of lymphoid follicles and aggregates might be due to a small number (two to three) of antral biopsy specimens. The higher rate of detection of lymphoid follicles and aggregates in the antrum in our study might be explained by the larger number (four) of antral specimens.

The highest prevalence and density of lymphoid follicles and aggregates was detected in 89.9\% and 0.82, respectively, in those patients in whom chronic gastritis as "background" with gastric ulcer. With increasing the number of biopsy specimens taken, higher prevalence and density of lymphoid follicles and aggregates might be
detected in gastric antral mucosa. Lymphoid follicles and aggregates in gastric antral mucosa might be found in 100% of *H. pylori*-positive patients if sufficient gastric biopsy specimens were examined, which is accordance with the finding of Genta et al. It is widely believed that the presence of lymphoid follicles and aggregates in gastric mucosa is a conspicuous pathologic feature of *H. pylori*-associated chronic gastritis.

It is remarkable considering the lack of lymphoid tissue in the normal gastric mucosa, and *H. pylori* infection is the major determinant of the development of gastric lymphoid tissue. Eradication of *H. pylori* infection result in decrease of lymphoid follicles and aggregates. In our study, only very few scattered lymphocytes were detected and no lymphoid tissue hyperplasia was found in the antral lamina propria in 190 biopsy specimens in the normal control group. Lymphoid follicles and aggregates in the gastric mucosa decreased quickly after successful eradication of *H. pylori* infection, which corresponded well to the previous data. No lymphoid tissue hyperplasia was detected in the gastric antral mucosa in 77.1% and 85.7% of patients with *H. pylori* negative one and two year after therapy in the present study. Our data further confirmed that the presence and degree of colonization with *H. pylori* is strongly correlated with the development and disappearance of lymphoid tissue in the stomach. After colonization of the gastric mucosa by *H. pylori*, close and persistent contact between the bacterium and the mucosa lead to a specific immune response. Lymphoid tissue hyperplasia is a specific immunological reaction to *H. pylori* infection. Previous studies have demonstrated that the proliferation of T-lymphocytes and macrophages inducing by cytotoxin and the production of *H. pylori*, and further release of several cytokines (IL-2, IL-6), that lead to the
proliferation of B-lymphocytes and the presence of lymphoid follicles and aggregates in infected gastric mucosa. The infiltration of T-lymphocytes decreased significantly when the colonization and density of *H. pylori* disappeared or decreased after eradication therapy, and then reduced to stimulate the proliferation of B-lymphocyte and disappearance of lymphoid follicles and aggregates in the gastric mucosa.

Our study confirmed the finding of Stolte and Eidt that there were significant correlations between lymphoid follicles and aggregates and the severity and activity of inflammation in the gastric mucosa. The development of lymphoid follicles and aggregates was maximal in the infected antral mucosa and occurred in the patients with severe chronic active gastritis. There was a progressive decline in the infiltration of chronic inflammatory cells in the antral mucosa with increasing degree of atrophy and intestinal metaplasia. With the regression of inflammation in the advanced stage of chronic gastritis, lesser numbers of lymphoid follicles and aggregates were detected. There were some main reasons for the loss of lymphoid follicles and aggregates. First, as *H. pylori* colonized only gastric epithelium, the organisms were absent from the areas of intestinal metaplasia that were usually present in atrophic stomach. Secondly, the hypochlorhydric environment is inimical to *H. pylori*. Thirdly, acidic glycoproteins secreted by metaplasia epithelium may provide a more hostile environment for *H. pylori* than the natural glycoproteins of normal mucosal layer. Fourthly, gastric epithelium and glandular tissue replaced by intestinal-type epithelium lead to lower numbers of specific receptor by *H. pylori*. Finally, intestinal epithelium is more resistant than gastric epithelium due to relatively higher concentration of specific Ig A. All above reasons might prohibit *H.
pylori to attach to intestinal-type cells.

In conclusion, there was closely correlation between lymphoid tissue hyperplasia and morphologic changes, such as the severity and activity of chronic gastritis, atrophy, intestinal metaplasia, as well as the colonization of *H. pylori*. Lymphoid follicles and aggregates due to *H. pylori* infection decreased quickly after eradication of the infection, and declined slightly and steadily but did not disappeared during the 2 years followed up period.

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**References**


