Mucosal change of the stomach with low-grade mucosa-associated lymphoid tissue lymphoma after eradication of Helicobacter pylori : Follow-up study of 48 cases

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Abstract: Low-grade mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach has been demonstrated to be closely linked to Helicobacter pylori (H. pylori) and to be frequently remissioned after the cure of H. pylori infection. Several previous studies have focused on proliferating lymphocytes but little is known about gastric epithelial change and the duration of the remission after the cure of H. pylori infection. We performed a long-term follow-up investigation on the effects of anti-H. pylori treatment on MALT lymphoma and chronic gastritis at the histologic and molecular levels. Forty-eight patients with low-grade gastric MALT lymphoma and 28 chronic gastritis patients in whom H. pylori infection was eradicated were studied. After eradication, 43 MALT lymphoma patients showed complete histologic remission and continuous remission was observed during follow-up for up to 43 months (mean, 17.8 months). As for epithelial changes after eradication, "emptiness of lamina propria" was more pronounced in the mucosa with MALT lymphoma than that with chronic gastritis, and its severity in MALT lymphoma cases significantly decreased during the observation period whereas the glandular area increased. Cystic change of the fundic gland also occurred more frequently in MALT lymphoma cases than chronic gastritis cases. B-cell clonality before eradication analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) was detected in almost all MALT lymphoma cases (43 cases), but rare in chronic gastritis cases (6 cases). After eradication, in spite of histologic regression, 21 MALT lymphoma patients had a persistent monoclonal population during the follow-up period. B-cell monoclonality preceding the malignant transformation was noted in 4 cases. These observations indicate that 1) complete histologic remission of low-grade gastric MALT lymphomas seems stable even if a monoclonal B cell population is detectable in some cases, 2) there may be a stage of disease where monoclonal B cells are present but there is no histologic evidence of MALT lymphoma, and 3) regenerative change of the damaged glands may occur in histologic regressed MALT lymphoma cases. J. Med. Invest. 47 : 36-46, 2000

Key words **:** *low-grade MALT lymphoma, epithelial change, empty lamina propria, B-cell clonality, H. pylori, eradication, long-term follow-up*

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INTRODUCTION

Malignant lymphomas of the mucosa-associated lymphoid tissue (MALT) type are an extra-nodal marginal-zone B-cell lymphoma (1, 2) and the most common of all gastric lymphomas (3). They may be

either low or high-grade (2). In low-grade MALT lymphomas of the stomach, small- to medium-sized irregular neoplastic B lymphoid cells, so-called centrocyte-like cells, densely infiltrate the lamina propria and surround, occasionally invading, the reactive-type follicles with germinal centers (1, 4). They also characteristically invade and destroy the epithelium of the gastric glands to form lymphoepithelial lesions (1, 4). High-grade MALT lymphomas, in which neoplastic B lymphoid cells are larger and apparently atypical, may either arise *de novo* or be transformed from low-grade MALT tumors (2).

It is currently accepted that Helicobacter pylori (H. pylori) plays a causative role not only in chronic active gastritis, peptic ulcer diseases and probably gastric carcinomas (5-7) but also in gastric low-grade MALT lymphomas (8-10). Clinical studies have reported high remission rates for low-grade MALT lymphomas after the cure of H. pylori infection (8-10). However, little is known about the duration of the remission because long-term follow-up study is limited (11). Moreover, data are also limited with regard to the epithelial changes in the stomach with MALT lymphomas during a long follow-up period.

The development of the polymerase chain reaction (PCR) provides a sensitive tool for the identification of neoplastic B cell populations by the analysis of immunoglobulin heavy chain (IgH) gene. However, the significance of the detection of B-cell monoclonality in gastric lymphoproliferative lesions by PCR remains controversial, because monoclonality can be detected not only in neoplastic (8-10) but also in histologically reactive lesions (10, 12, 13). Its clinicopathological significance has yet to be established.

The aims of this histologic and molecular study on H. pylori-associated gastric lymphoproliferative diseases including low-grade MALT lymphomas and chronic gastritis are 1) to assess the duration of the remission, 2) to clarify the relationship between histologic change and B-cell clonality, and 3) to investigate the epithelial changes over a long-term follow-up period after eradication of H. pylori.

MATERIALS AND METHODS

Patients

Seventy-six patients with H. pylori-infected gastric lymphoproliferative lesions, either chronic gastritis or low-grade MALT lymphoma, were studied. All patients had initially presented with nonspecific upper gastrointestinal symptoms. All previous clinical records and histopathologic slides were checked if previous history presented. In MALT lymphoma cases, the involvement of lymph nodes or other organs was assessed by physical examination, routine laboratory tests, chest x-ray, thoracic and abdominal CT and abdominal ultrasound analysis, and the lesion was confined to the stomach.

Tissue specimens

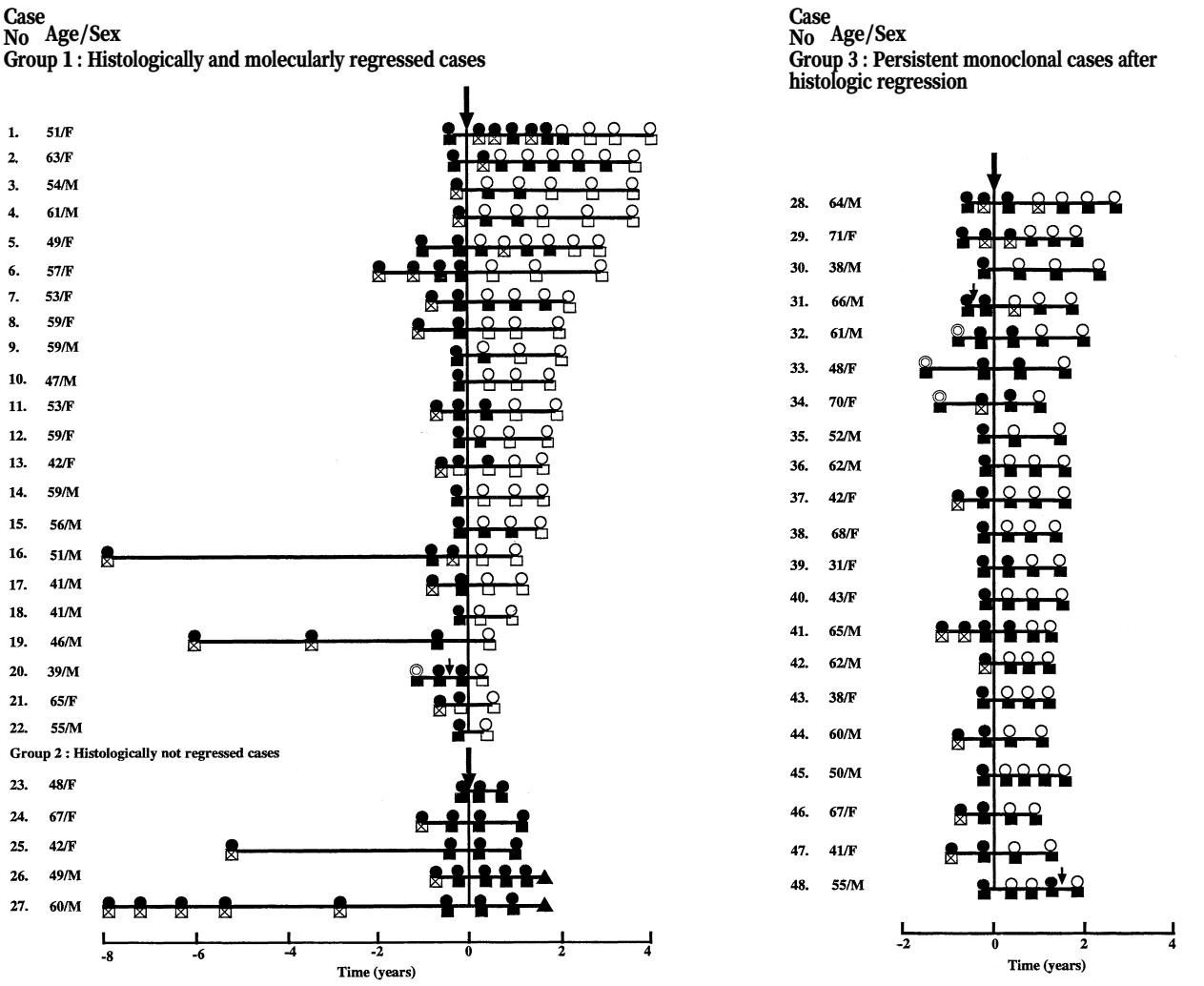
On each occasion, the endoscopy was undertaken by the same endoscopist (Y.U.). The endoscopic findings alone could not discriminate between chronic gastritis and low-grade MALT lymphoma before eradication. Six to ten biopsy specimens were taken from suspicious areas for histology and two others for reverse transcriptase-polymerase chain reaction (RT-PCR) study. The biopsy specimens were immediately fixed with 10% neutral buffered formalin and kept at room temperature for one day. Thin sections were routinely prepared and stained for hematoxylin and eosin and Giemsa. Biopsy materials for RT-PCR were stored without fixation at -80°C until use.

Eradication and follow-up

All 76 patients received anti-H. pylori therapy consisting of a combination of lansoprazole (30 mg/day), metronidazole (500 mg/day) and amoxicillin (1.5 g/day) for 7 to 14 days. All patients underwent endoscopy two to four months after completion of the eradication therapy, and histologic and molecular analysis was done. Subsequently MALT lymphoma patients underwent repeated endoscopic, histologic and molecular analysis at approximately six-month intervals for up to 45 months after anti-H. pylori therapy (mean, 20.2 months) (Fig.1), although 2 of them (Cases 26 and 27 in Fig.1) were referred for surgical treatment. Patients whose initial biopsies were assessed as showing a reactive lymphoid infiltrate (chronic gastritis) were followed up only once or twice for up to 33 months (mean, 8.4 months) after eradication treatment.

Histologic criteria

Histopathologic grading for differential diagnosis of gastric lymphoid proliferative lesions was done using the scoring system of Wotherspoon *et al*. (8). Briefly, grades up to 3 were considered reactive (chronic gastritis) and grades 4 and 5 neoplastic



↓-H. pylori eradication therapy was given ; ●-MALT lymphoma, histologically ; ○-Histological regression occurred ; ◎-Chronic gastritis, histologically ; ■-Monoclonal by PCR ; □-Polyclonal by PCR ; ⊠-PCR was not performed ; ▲-Surgical treatment was given. For example, ● means histologically MALT lymphoma and monoclonal by PCR

Fig.1. Histopathologic and RT-PCR results during follow-up for 48 low-grade gastric MALT lymphoma patients.

(low-grade MALT lymphoma). The criteria for the diagnosis of low-grade gastric MALT lymphomas were the detection of lymphoepithelial destruction, and the presence of dense diffuse infiltrates of centrocyte-like cells in lamina propria and germinal centers. Presence or absence of lymphoepithelial lesions, lymphoid aggregates and lymphoid follicles was evaluated for differential diagnosis.

Measurement of the degree of "empty lamina propria"

Histologic change in the degree of so-called" empty lamina propria "was morphometrically followed up in 10 selected MALT lymphoma cases for up to 37 months. Nine chronic gastritis cases were also studied as a control for up to 22 months. This change was characterized by loose connective tissue with scattered plasma cells, an absence of

Table 1. Sequences of primers for PCR

(For immunoglobulin heavy chain)
(Upper-stream)
VH1LS ; 5-'CAT GGA CTG GAC CTG GAG G-3 '
VH2+4LS ; 5-'CAT GAA ACA CCT GTG GTT CTT-3 '
VH3LS ; 5-'GGG CTG AGC CTG GGT TTT CCT T-3 '
VH5LS ; 5-'GGG GTC AAC CGC CAT CCT-3 '
VH6LS ; 5-'TCT GTC TCC TTC CTC ATC TTC-3 '
(Down-stream)
Cμ ; 5-'GGT TGG GGC GGA TGC ACT-3 '
(For nested)
VHFR2 ; 5-'GC(C/T) (C/T)CC GG(A/G) AA(A/G) (A/G)GT CTG GAG TGG-3 '
(For GAPDH as an internal control)
(Upper-stream)
GAPDH-UP ; 5-'GAAATCCCATCACCATCTTCCAGG-3 '
(Down-stream)
GAPDH-DN ; 5-'CATGTGGGCCATGAGGTCCACCAC-3 '

organized collections of lymphoid cells, and a loss of gastric glands (10). Measurement of the" empty lamina propria "was done as follows : the 3 most prominent areas were selected in each case and photographed at a magnification of 160 times under a microscope. The field of one photograph (110.88 cm^2) was divided into 176 squares and the number of squares containing" empty lamina propria "was counted. The mean value (%," empty lamina propria " area/total area) was calculated by analyzing 3 photographs.

Immunohistochemistry

An immunohistochemical study was done on 20 MALT lymphoma and 5 chronic gastritis patients. According to previously described methods (14), the procedure was performed with L26 (CD20, 1 : 100 ; DAKO, Carpinteria, USA) for B lymphocytes and CD3 (1 : 100 ; DAKO) for T lymphocytes.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR for detection of rearranged IgH genes in proliferating lymphoid cells was performed in 45 MALT lymphoma and 20 chronic gastritis cases before eradication. Materials in the remaining 3 MALT lymphoma cases were not available. After eradication, biopsy materials for RT-PCR were available in all 48 MALT lymphoma cases.

RNA Extraction : Total RNA was extracted from the samples by the modified acid-guanidinium-thiocyanate-phenol-chloroform (AGPC) method (15) using ISOGEN RNA extracting mixture (Nippon Gene, Toyama, Japan) according to the manufacturer's recommendations. When intact 28S and 18S ribosomal RNAs were visualized on agarose gel, total RNA from the samples was subjected to RT- PCR.

RT-PCR : In order to identify monoclonal B cell proliferation in the gastric lesions, we amplified the entire VH region of IgH cDNA by RT-PCR. For reverse-transcription, 5 μg of total RNA was incubated with M-MLV (Life Technologies, Inc. Gaithersburg, USA) and a dNTP mixture (500 μM each ; Life Technologies, Inc.) at 42°C for 60 min using a random primer (5 μM ; Life Technologies, Inc.) in 20 μl of a reaction mixture. Subsequently, 1 μl of the products was subjected to PCR amplification (16). We used a mixture of five leader sequence primers (VH1LS, VH2+4LS, VH3LS, VH5LS and VH6LS ; Table1) corresponding to 6 VH regions in IgH genes as an upper-stream primer, and a Cμ sequence (Table 1) in the CH 1 region as a down-stream primer. We used a pair of primers for GAPDH gene (GAPDH-UP and GAPDH-DN ; Table 1) as an internal control for an adequate PCR reaction. PCR was performed as follows ; the final concentration of dNTPs and primers in the reaction mixture is 200 μM and 1 μM, respectively. Taq DNA polymerase (Takara, Shiga, Japan) was added to the mixture at a final concentration of 0.05 U/μl and the reaction was carried out in Takara Thermal Cycler MP (Takara) under the following conditions ; 94°C for 3 min and then 94°C for 1 min, 55°C for 1.5 min, and 72°C for 2.5 min for the desired number of cycles and an extension of 72°C for 4 min. In some cases, we performed semi-nested PCR to confirm the reliability and the reproducibility of our experiment using the same upper-stream primer mixture for first PCR and a new down-stream primer (VHFR2 ; Table 1) at the FR2 region in IgH gene. Then, PCR amplification was done under the conditions described above. The PCR products were electrophoresed in 1.7% agarose gel or 5% polyacrylamide gel and visualized under ultraviolet light after ethidium bromide staining. The size of PCR products was evaluated by comparison with either Pvu2 digested pBK-CMV or Hap2 digested pUC19.

We used one B cell lymphoma sample as a positive control and mononuclear cells in peripheral blood prepared from a healthy volunteer as a negative control. To eliminate the possibility of the contamination of positive samples in the reagents, one sample without template was also added in each experiment. For each case, the reactions were done at least in duplicate, and only the cases with a dominant band of identically sized fragments were considered to contain a monoclonal B cell population.

Statistical analysis

Statistical differences were evaluated using Student's t-test and Paired t-test, as applicable. A value of $P<0.05$ was considered statistically significant.

RESULTS

Histopathology

As defined in Materials and Methods, histologic evaluation of 76 patients distinguished a reactive lymphoid infiltrate (score 2-3) in 28 cases (16 males and 12 females) and features suggestive or diagnostic low-grade MALT lymphoma (score 4-5) in 48 (25

males and 23 females). Patients with chronic gastritis presented at a mean age of 45.2 years (range 32-55) and low-grade MALT lymphoma at 49.4 years (range 31-71) (Table 2). H. pylori organisms were identified in the initial biopsy specimens in all cases by examination of the Giemsa stained sections.

Clinical and histologic comparative results of 76 patients are shown in Tables 2, 3 and 4. In cases of chronic gastritis, the mononuclear infiltrate consisted of small lymphocytes with round nuclei and plasma cells, but few or no centrocyte-like cells (Fig.2). As shown in Table 3, the infiltrate was either mild (9 cases), moderate (18 cases) or severe (1 case). Lymphocytes infiltrating epithelium but not lymphoepithelial lesion were present in 9 cases (32%), lymphoid aggregates in 23 (82%) and follicles with germinal centers in 8 (28%). Eradication of H. pylori was achieved in all chronic gastritis cases. These patients were followed up for a mean of 8.4 months (range 2-33) after H. pylori treatment. A remarkable decrease in the lymphoid infiltrate was observed at the first post-therapy evaluation (within 2 months) ; lymphoid aggregates were seen in 9 cases (32%) and lymphoid follicle in one case (3%) (Table 3).

In all 48 MALT lymphoma cases, centrocyte-like cells, lymphoepithelial lesions and lymphoid follicle

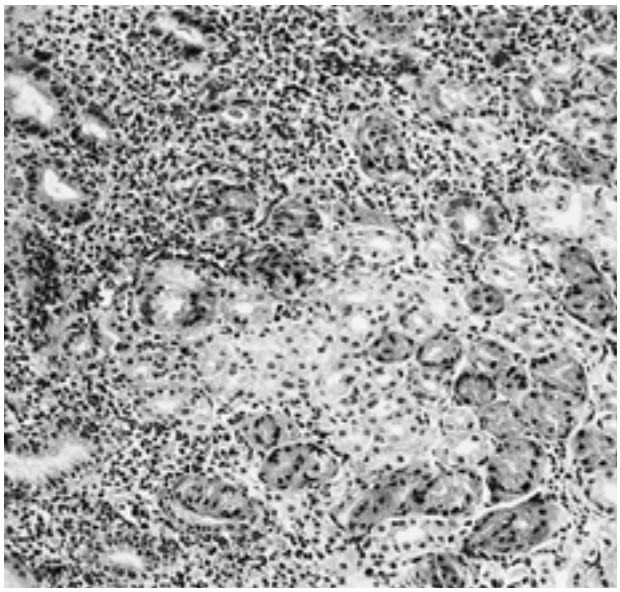


Fig. 2. Chronic gastritis. Small lymphocytes with round nuclei, plasma cells and rare centrocyte-like cells infiltrate the lamina propria. (Hematoxylin-eosin stain ; original magnification×150)

Table 2. Clinical profile in the cases of chronic gastritis and MALT lymphoma

	Number of cases	Sex		Age (year) mean (range)	Follow-up period (month) mean (range)	Follow-up period after eradication (month) mean (range)
		Male	Female			
Chronic gastritis	28	16	12	45.2 (32-55)	10.6 (4-36)	8.4 (2-33)
MALT lymphoma	48	25	23	49.4 (31-71)	28.9 (4-113)	20.2 (3-45)

Table 3. Comparison of the histopathologic and molecular findings between patients with chronic gastritis and MALT lymphoma before and after H. pylori eradication

Type of lesion	Stage	Chronic inflammation Mild	Moderate	Severe	Lymphoid aggregates		Lymphoid follicles		LEL	Cystic change	B cell★ monoclonality	
Chronic gastritis (n=28)	Before ER	9	18	1	23	*	8	**	0	0	6	NS
	After ER	28	0	0	9		1		0	4	3	
MALT lymphoma (n=48)	Before ER	0	2	46	48	*	48	*	48	0	43	*
	After ER											
	HR (n=43)	36	7	0	23		8		0	28	21	
	HNR (n=5)		5		5		5		5		5	

LEL : Lymphoepithelial lesion, ER : Eradication, HR : Histologically regressed cases, HNR : Histologically non- regressed cases,
★ : Twenty chronic gastritis and 45 MALT lymphoma cases were analyzed for B cell monoclonality before eradication,
NS : Not significant
*p<0.001, **p<0.05, by Paired t-test.

were present (Fig. 3). Eradication of H. pylori was achieved in all cases (2 after a second treatment course). Patients were followed up for a mean of 20.2 months (range 3-45) after H. pylori treatment and 17.8 months (range 0-43) after histologic remission. As shown in Fig.1, histologic regression was seen in 43 of 48 patients (89%) and was evident in the first post-treatment biopsy performed 2 to 4 months after eradication in 32 cases (72%) (Fig. 4a). Among the remaining 11 cases, histologic regression was observed 4 to 8 months later in 10 cases and 22 months later in one case (Case 1). Small lymphocyte clusters without centrocyte-like cells were seen in 23 cases (48%), and lymphoid follicles in 8 cases (23%) (Table 3). In one patient (Case 48), relapse of MALT lymphoma was associated with reinfection. This reinfection was retreated and the patient was then in remission stage.

Low-grade MALT lymphoma was also diagnosed by retrospective re-evaluation of histology which was done for biopsy specimens taken from 5 patients 2, 5, 6, 8 and 8 years before the present study, respectively (Cases 6, 16, 19, 25 and 27 in Fig.1). Three of these 5 patients' histologic regression occurred after eradication (Fig.1).

Among 48 patients with low-grade MALT lymphoma, 5 patients showed no histologic regression after H. pylori treatment. Two were referred for surgical treatment and histologic analysis revealed a component of high-grade lymphoma in the resected stomach (Cases 26 and 27 in Fig.1). The remaining 3 patients were followed up for 6, 10 and 13 months and showed no histologic change (Cases 23-25 in Fig.1).

We followed up the change in the degree of "empty lamina propria" (Table 4). In chronic gastritis cases, there was no significant difference in the degree of empty lamina propria between initial observation (mean, 57%) and final observation (mean, 54%) (P=0.7338). On the other hand, in MALT

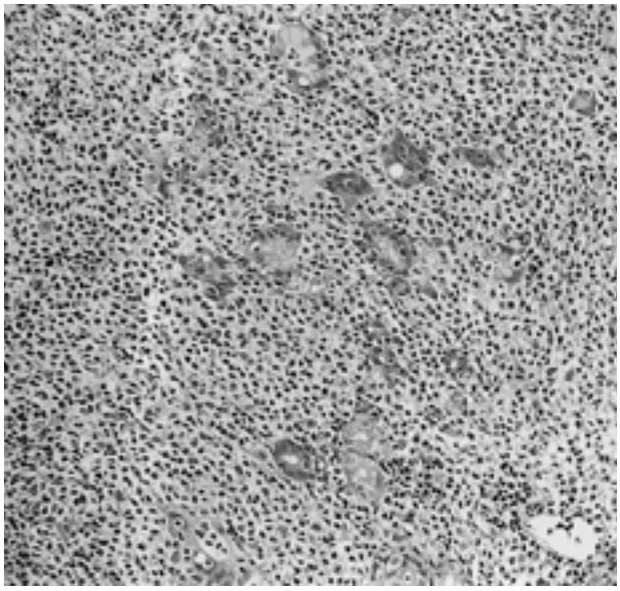


Fig. 3. Low-grade gastric MALT lymphoma. Mucosal infiltration by centrocyte-like cells that form prominent lymphoepithelial lesions (score 5) (Hematoxylin-eosin stain ; original magnification×150)

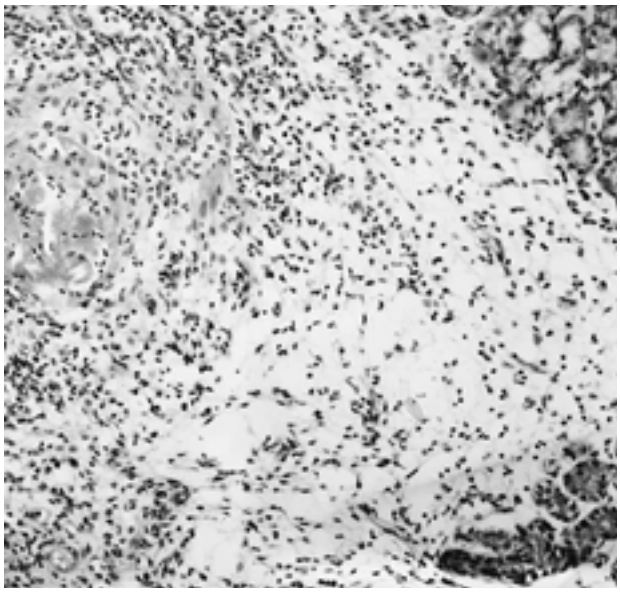


Fig. 4a. Low-grade gastric MALT lymphoma. Five months after eradication, there are occasional small lymphoid aggregates with loss of gastric glands within an empty-appearing lamina propria. (Hematoxylin-eosin stain ; original magnification×150)

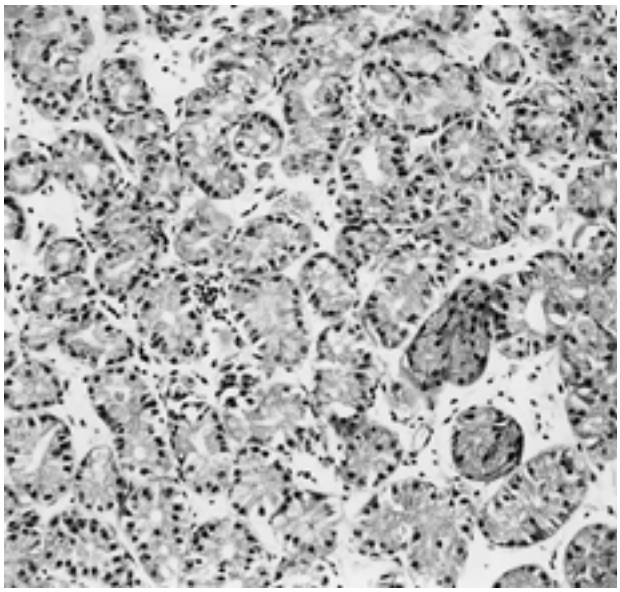


Fig. 4b. Forty-five months after eradication, emptiness of the lamina propria has markedly decreased and the glandular area increased. (Hematoxylin-eosin stain ; original magnification×150)

lymphoma cases, there was a significant difference in the degree of empty lamina propria between initial and final observation (P=0.0002) (Fig. 4b). It decreased gradually ; in the first observation (4 months of post-eradication) the mean value was 80%, in 12 months 67%, in 24 months 62% and in 28.2 months 59% (Table 4).

After eradication, dilatation of the fundic gland was seen in 4 chronic gastritis cases (14%) and 28 MALT lymphoma cases (58%). Dilatated glands of various size were surrounded by normal fundic gland and lined by parietal cells, chief cells and mucous secreting cells (Fig. 5). Lingular projection of parietal cells was also seen in 4 MALT lymphoma cases.

Immunohistochemistry

Immunohistochemical analysis confirmed the B-cell predominance within the lymphoid infiltrates ; the majority were L26 positive-B cells but CD3 positive-T cells were also present. Lymphoid aggregates and germinal centers which were located deep in the mucosa were of B lineage. Lymphocytes in lymphoepithelial lesions were phenotyped as B lineage cells but a few T cells were also noted.

Table 4. Degree of empty lamina propria (%) during follow-up

	Month of follow-up (mean)			
	4 m	12 m	24 m	28.2 m
Chronic gastritis (n=9)	57	54		
	NS			
MALT lymphoma (n=10)	80	67	62	59
	P=0.0003			
	P=0.0005			
	P=0.0002			

NS : Not significant

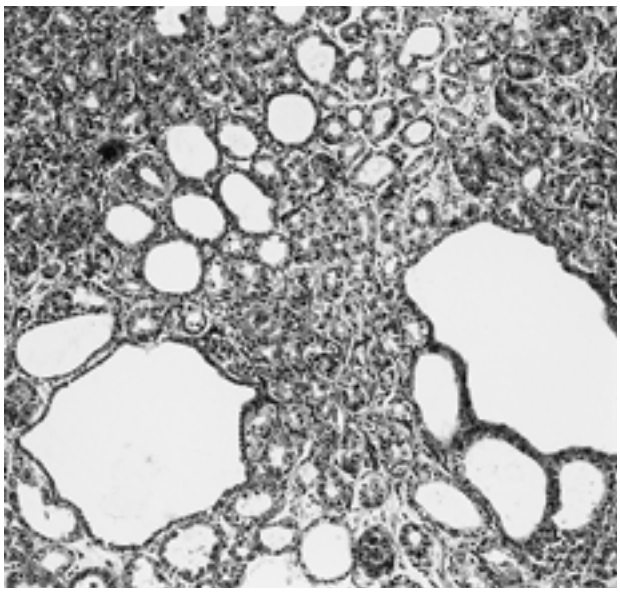


Fig. 5. Low-grade gastric MALT lymphoma. After eradication, multiple glandular cysts are seen in the corpus mucosa. (Hematoxylin-eosin stain ; original magnification×87)

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Among 20 chronic gastritis cases in which RT-PCR was performed, in 6 there was evidence of a clonal population before H. pylori treatment. Follow-up biopsies after eradication (mean 15.1 months ; range 8-33) showed continued absence of histologic features of lymphoma but RT-PCR showed persistence of a clonal band in 3 of these 6 cases.

As shown in Fig.1, RT-PCR was performed in 45 MALT lymphoma cases before eradication and a detected monoclonal band in 43 cases and polyclonal band in 2 cases (Cases 13 and 21). After eradication, RT-PCR was performed in 48 MALT lymphoma cases. In the first post-treatment analysis (2 to 4 months after eradication), a polyclonal band was detected in 12 cases and monoclonal band in 36 cases. During the follow-up period, in 10 of these 36 cases there was a change to a polyclonal band while in 26 cases (21 histologically regressed cases and 5 non-regressed cases), the monoclonal band persisted.

In 4 cases (Cases 20 and 32-34 in Fig.1), in spite of histologic features of a reactive lymphoid infiltrate (score 2), RT-PCR detected a monoclonal population in the first biopsy. Rebiopsy, done 3 to 19 months later but before eradication, revealed histologic evidence of MALT lymphoma.

DISCUSSION

It is now commonly accepted that gastric B-cell MALT lymphoma is, in most cases, a sequela of H. pylori-induced, T-cell mediated chronic gastritis (17). The finding of regression of MALT lymphoma brought about by eradication of H. pylori infection is an important step in establishing a role for H. pylori in the events leading to MALT lymphoma (8-10). Further evidence of a role for H. pylori came from case reports in which reinfection with H. pylori resulted in a relapse of MALT lymphoma (18, 19).

Epithelial changes of regressed patients with

MALT lymphoma during long-term follow-up have not been evaluated in other trials. In the present study, we focused on particularly empty lamina propria that was more pronounced in the case of MALT lymphoma than chronic gastritis. The demonstration of a more pronounced empty lamina propria in MALT lymphoma cases indicates possible dense lymphoid infiltration. During the follow-up period (mean 28.2 months ; range 13-37), the area of empty lamina propria decreased and the glandular area increased which was statistically significant. These results suggest that regenerative change of the damaged glands caused by lymphoepithelial lesions may occur in histologically regressed cases. Reversal of glandular atrophy in patients with chronic gastritis after eradication has been recently reported but the results in these reports have been conflicting. While in a few studies reversal of atrophy was found in all or almost all atrophic gastritis patients examined (20-22), only a few or no patients showed reversal of atrophy in other studies (23, 24). This discrepancy may be due to different locations and grades of atrophic gastritis. Reappearance of parietal and chief cells following corticosteroid and anti-ulcer drug sulglycotide treatment in patients with atrophic gastritis has been reported (25-27). These results further support our speculation that regeneration takes place after H. pylori eradication. In the chronic gastritis cases, the gastric gland also recovered but the extent of the recovery was not significant. This may be due to the less dense lymphoid infiltrate in our study. The relatively short-term follow-up (mean 12.2 months ; range 12-33) may also explain the discrepancy (20-22).

The presence of dilated glands and intramucosal cysts lined by parietal, mucus and chief cells is the histologic hallmark of fundic gland polyp (28). In our series, there were lesions where histology fulfilled the criteria for the diagnosis of fundic gland polyps but endoscopic features were absent. The fact that cystic change of the fundic gland was seen in 28 MALT lymphoma cases (58%) and in 4 chronic gastritis cases (14%) after H. pylori eradication suggests a relationship between eradication therapy and cystic change of the fundic gland. Recent reports have found an association between fundic gland polyp and chronic omeprazole use (29, 30). Ang *et al*. have recently speculated that cysts, parietal cell hypertrophy and lingular projections of parietal cells are likely to be forerunners to the fundic gland polyps in some patients receiving long-term omeprazole therapy (31). However, in our studies we found lingular projection of parietal cells only in 4 cases. The reason for this discrepancy may be that the treatment period (10-14 days) in our study was shorter than those in the previous study groups.

For the differential diagnosis of reactive and neoplastic lymphoid proliferation in the gastric mucosa, most reports have focused on the detection of monoclonality (8-10). However, the significance of the detection of B-cell monoclonality by PCR remains controversial, because monoclonality is detected not only in neoplastic (8-10) but also in histologically reactive lesions (10, 12, 13). In our series, in fact, 10 cases showed histologic features of a reactive lymphoid infiltrate with a PCR detectable monoclonal population. In the absence of histologic signs of low-grade gastric MALT lymphoma, the presence of monoclonal B cells may indicate a special or peculiar form of gastritis with a monoclonal evolution of B cells or suspicious borderline lesion (32). Whether these B cells are prone to evolve into early gastric MALT lymphoma can only be speculated presently. During the follow-up period, histologic features of lymphoma did not develop in the 6 patients eradicated of chronic gastritis with monoclonality in our series and in others (10, 13). In low-grade MALT lymphoma, the cure of H. pylori infection induces complete histologic remission in the majority of cases and the relapse rate is also low. So it is natural that after eradication of H. pylori, histologic features of monoclonal chronic gastritis may be sustained. In contrast, among those 10 monoclonal chronic gastritis cases, histologic evidence of lymphoma was found in 4 cases during follow-up. Before eradication of H. pylori, we performed a second histologic examination in these 4 cases. In addition to our series, MALT lymphoma developed in 14 monoclonal chronic gastritis cases after more than 1 year of follow-up without eradication of H. pylori (33). A PCR detectable population in the absence of histologic features of lymphoma might therefore represent a peculiar tumor progression stage when the clonal population is large enough to be detected by PCR but not by histology. It seems that monoclonality precedes histologic neoplastic transformation and that a diagnosis of B-cell malignancy should not be based solely on the detection of a monoclonal rearrangement of the IgH gene by PCR.

In 21 cases with low-grade MALT lymphoma, there was persistent molecular evidence of a clonal population after histologic regression. It is not clear whether these patients still suffer from the disease

or monoclonality only indicates the presence of benign memory B-cell precursors of the malignant clone (32). The patients with monoclonal PCR bands may be more likely to have a relapse of their disease. There was only one case of relapse in our series, and the patient was monoclonal before the relapse was detected. The rapid recurrence of MALT lymphoma in our case could be explained by regression of the pathologic features of MALT lymphoma caused by H. pylori eradication and persistence of the neoplastic cells in a latent form. Subsequently, re-exposure to the H. pylori might result in reactivation of the neoplastic cells and rapid relapse of the MALT lymphoma (18). It is difficult to draw any conclusion at present. Follow-up studies will answer the question of whether patients with monoclonal B cells are at high risk of relapse of the disease.

Low-grade MALT lymphoma was diagnosed by retrospective re-evaluation of histology in 5 cases and a spontaneous cure was not seen in any of these cases within this period. We predict that a natural cure is possible. A decrease in H. pylori in the stomach has been considered to be caused by mucosal atrophy with intestinal metaplasia (34) and mucosal destruction (33). We speculate that intestinal metaplasia creates a hostile environment for H. pylori which can lead to a natural cure although it may take a long time.

Most of the studies published to date have not described the duration of the remission of MALT lymphomas after the cure of H. pylori infection. Complete remissions were obtained in 43 of 48 patients (89%) in our series and continued throughout the follow-up period (mean 17.8 months ; range 0-43), suggesting that most patients who achieve a complete remission do not relapse within a short period of time. Recently, one follow-up study by Neubauer *et al*. revealed 40 patients in complete remission for a median of 14 months similar to our analysis (11). Only one relapse of MALT lymphoma was observed in our series but it was associated with reinfection of H. pylori. Case reports on relapse of the low-grade MALT lymphoma are very rare and in most of the cases relapse of MALT lymphoma was associated with reinfection of H. pylori (18, 19).

In 5 patients, no histologic change of low-grade MALT lymphoma was seen after cure of H. pylori infection. Of these 5 patients, 2 underwent surgery and a high-grade lymphoma component was detected which was not detected in previous gastric biopsy specimens. Gastric MALT lymphoma may be the result of a multistep process in which a T-cell-driven B-cell growth first leads to monoclonal B-cell proliferation (17), and then to the typical lymphoepithelial lesion (8), then a low-grade B-cell clone evolves that may not depend on T-cell help (35) and finally a high-grade B-cell clone can be found which may be dependent upon additional genetic changes such as p53 alteration (36) and bcl-6 protein (37). In a recent review by Isaacson (35), it was pointed out that the regressed low-grade MALT lymphomas following the eradication of H. pylori were all in an early phase. Patients not responding to cure of H. pylori infection may be in a progressive stage of the disease.

In conclusion, our results suggest that 1) regenerative change of the gastric mucosa occurs in the histologically regressed MALT lymphoma, 2) genetic monoclonality precedes histologic neoplastic transformation, and 3) long-lasting remissions can be acquired after the cure of H. pylori infection in patients with early stage low-grade gastric MALT lymphoma. Due to the indolent nature of low-grade MALT lymphoma, the possibility of reinfection and subsequent relapse of MALT lymphoma requires regular follow-up.

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