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B-Cell Monoclonality Precedes the Development of Gastric MALT Lymphoma in *Helicobacter pylori*-Associated Chronic Gastritis

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Little is known about the temporal changes in *Helicobacter pylori* density and B-cell clonality during the evolution from chronic gastritis to gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Biopsied specimens from 28 patients with chronic gastritis who developed gastric MALT lymphoma (group A) and from 24 similar patients who did not (group B) during an equivalent follow-up period (mean, 42 months) were retrospectively scored for histological features of MALT lymphoma (0 to 5) and *H. pylori* density (0 to 3). B-cell clonality was analyzed by polymerase chain reaction (PCR). During the observation period, the *H. pylori* density in group A decreased significantly in comparison with group B; the mean change in *H. pylori* density (final minus initial density) per 1000 days was −1.4 for group A and +0.2 for group B ($P < 0.005$). Monoclonality was detected more frequently in group A (79%) than in group B (21%; $P < 0.005$), and it preceded the histological evidence of malignant transformation in 64% of those patients who showed monoclonality in group A. These results suggest that *H. pylori* is thus more closely associated with the precursor or initial phase in the genesis of gastric MALT lymphoma than with the later phase, as its density decreases as the tumor progresses. The detection of B-cell monoclonality by PCR is thus of possible use for predicting the histological genesis of gastric lymphoma. *(Am J Pathol 1998, 152:1271–1279)*

It is currently accepted that *Helicobacter pylori* plays a causative role not only in chronic active gastritis, peptic ulcer diseases, and gastric carcinomas[1–3] but also in gastric lymphomas, especially the mucosa-associated lymphoid tissue (MALT) type.[4–11] Our previous large study based on gastrectomy specimens detected *H. pylori* in 90% of the gastric lymphomas restricted to the mucosa and the superficial portion of the submucosa, although it was found in only 50% of those that invaded the deep portion of the submucosa or beyond, thus suggesting that *H. pylori* may disappear during the progression of lymphoma.[11] As yet, however, little is known about the temporal changes in the quantity of *H. pylori* that take place during the genesis of gastric lymphoma.

In addition, the detection of a monoclonal rearrangement of the immunoglobulin heavy chain (IgH) gene in small biopsy specimens by the polymerase chain reaction (PCR) technique has been reported as useful for identifying neoplastic B-cell populations.[7,10,12–14] However, B-cell monoclonality is sometimes detected in histologically reactive lesions by PCR,[14–16] and its clinicopathological significance has yet to be established.

The aims of the current study are 1) to delineate the dynamic changes in *H. pylori* density during the evolution of gastric MALT lymphoma from chronic gastritis and 2) to assess the significance of the detection of B-cell clonality in gastric lymphoproliferative diseases. For these purposes, we retrospectively studied the biopsied specimens of 28 patients with chronic gastritis or reactive lymphoid hyperplasia who developed gastric MALT lymphoma and those of 24 similar patients who did not during an equivalent long-term observation period.

Materials and Methods

Patients

All of the patients in this study were retrospectively selected from among the upper gastrointestinal endoscopic examination files of either Kyushu University Hospital or Matsuyama Red Cross Hospital between 1977 and 1994. The case group (group A) consisted of 28 Japanese patients with chronic gastritis who had been followed up both endoscopically and histologically for more than 1

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Table 1. Comparison of the Clinical Findings between Group A and Group B

	n (%)*		Initial age (years)†		Follow-up period (months)†		Number of endoscopic examinations†		Number of examined specimens‡		Site of lesion (*n* (%))§		
	Male	Female	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Upper third	Middle third	Lower third
Group A (*n* = 28)	11 (39)	17 (61)	54 ± 13	18–81	40 ± 38	12–147	6.5 ± 4.0	3–21	33 ± 37	9–209	12 (43)	12 (43)	4 (14)
Group B (*n* = 24)	10 (42)	14 (58)	54 ± 15	27–78	43 ± 43	12–189	4.3 ± 2.0	3–13	23 ± 21	6–96	4 (17)	15 (63)	5 (21)

*Differences between the two groups were not significant by χ^2 test.
†Differences between the two groups were not significant by Mann-Whitney *U* test.
‡P = 0.120 by Mann-Whitney *U* test.
§P = 0.082 by χ^2 test.

year and who thereafter ultimately developed gastric B-cell MALT lymphoma. We designated 1 year as the minimal observation period over which to observe any dynamic changes in the factors studied. This would minimize the possibility of overlooking the presence of lymphoma at the beginning of the follow-up period. The control group (group B) included 24 patients with chronic gastritis who had also been followed up for more than 1 year but who demonstrated no evidence of gastric lymphoma by the end of the observation period. According to the histological scoring system for the diagnosis of MALT lymphoma proposed by Wotherspoon et al,[7] all patients showed at least a score of 2 (chronic active gastritis with florid lymphoid follicle formation). None of them had undergone antibiotic treatment for the eradication of *H. pylori* by the end of the observation period. As shown in Table 1, no significant differences were observed between groups A and B regarding sex distribution, age at initial observation, the period of follow-up (40 ± 38 months for group A and 43 ± 43 months for group B; mean ± SD), the number of endoscopic examinations and biopsied specimens, or the site of the lesions.

At the end of the follow-up in group A, the development of B-cell MALT lymphoma was confirmed based on histological and immunohistochemical examinations of biopsied or endoscopically resected specimens or surgical materials. The criteria for low-grade MALT lymphoma were defined in accordance with those established by Isaacson et al[17] as a diffuse proliferation of centrocyte-like cells with tissue destruction and the formation of lymphoepithelial lesions (these criteria are identical to those with a score of 5 according to the histological scoring system by Wotherspoon et al[7]). Immunophenotyping (B-cell/T-cell) and immunoglobulin light chain restriction were assessed using monoclonal antibodies, including kappa, lambda, CD20 (L26), CD45RO (UCHL1, OPD4), and a polyclonal antibody CD3 (Dako, Glostrup, Denmark), as described in a previous report.[18] According to the criteria of Isaacson et al,[17–19] 19 of 28 lymphomas (68%) were classified as low-grade MALT lymphoma, 6 (21%) as low-grade MALT lymphoma with a focal high-grade component, and 3 (11%) as high-grade MALT lymphoma with a low-grade component. Twenty-five patients were treated by either a total or subtotal gastrectomy and three by eradication of *H. pylori*. The degree of the depth of tumor invasion was as follows: nine lymphomas were restricted to the mucosa and the superficial portion of the submucosa[11] (including three unresected lymphomas assessed by endosonography alone[20]), nine had massively invaded the deep portion of the submucosa, five involved the muscularis propria, and five had reached the subserosa or serosa. All patients remained alive without any evidence of lymphoma recurrence after treatment, except for two patients who had died of other diseases 12 and 26 months after undergoing gastrectomy.

Assessment of Histology and H. pylori *Density*

All examined materials were obtained by endoscopic biopsy or endoscopic mucosal resection.[21] In each patient, the biopsies were taken from not only endoscopically affected lesions but also unaffected antrum and corpus, at least during both the initial and final endoscopic examinations. These tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, and routinely stained with hematoxylin and eosin (H&E). All of these H&E-stained specimens along with the later immunohistochemical specimens were reviewed separately by two observers (S. Nakamura and T. Yao), and a consensus was reached in all patients.

To assess any alterations in the histological appearance that were suggestive of malignant potential in subsequent specimens, the diagnosis of gastric MALT lymphoma was objectively scored using a previously published system[7] (scored from 0 to 5) in which scores of up to 3 were considered reactive and scores of 4 or 5 were considered neoplastic.[7,8,10]

The presence of *H. pylori* was studied on formalin-fixed and paraffin-embedded specimens by H&E stain as well as immunohistochemical staining with a polyclonal rabbit anti-*H. pylori* antibody B471 (Dako) using the streptavidin-biotin-peroxidase complex technique, as described in a previous report.[11] To assess the quantity of *H. pylori*, the *H. pylori* density was scored on the sections immunostained with B471, in which the unaffected mucosal epithelia remained and the largest amount of *H. pylori* was noted, from grade 0 to grade 3, according to the updated Sydney System[22]: grade 0, no bacteria; grade 1, a mild amount of bacteria; grade 2, a moderate amount of bacteria; and grade 3, a marked amount of bacteria.[11]

Table 2. Comparison of the Histopathological and Molecular Findings between Group A and Group B

	Initial observations			Final observations				
	Lymphoma score	*H. pylori* density	*H. pylori* positivity (*n* (%))	Lymphoma score	*H. pylori* density	*H. pylori* positivity (*n* (%))	Change in *H. pylori* density in 10^3 days	B cell monoclonality (*n* (%))
Group A ($n = 28$)	2.0 ± 1.0	1.8 ± 1.0	24 (86)	4.5 ± 0.5	0.7 ± 0.9	17 (61)	−1.4 ± 1.7	22 (79)
Group B ($n = 24$)	2.1 ± 1.1*	1.8 ± 0.8*	23 (96)†	1.8 ± 0.8‡	1.9 ± 1.0‡	22 (92)§	+0.2 ± 2.2¶	5 (21)‖

*The differences were not significant by Mann-Whitney *U* test.
†The difference was not significant by χ^2 test.
‡$P < 0.001$ by Mann-Whitney *U* test.
§$P < 0.05$ by χ^2 test.
¶$P < 0.005$ by Mann-Whitney *U* test.
‖$P < 0.005$ by χ^2 test.

For a detailed histological evaluation, the degrees of neutrophilic activity, chronic inflammation, and intestinal metaplasia were also scored on H&E-stained sections from grade 0 to grade 3, according to the updated Sydney System.[22] In addition, the presence of lymphoepithelial lesions and germinal centers was also evaluated.[23]

Polymerase Chain Reaction

To detect B-cell monoclonality, the IgH gene rearrangement was analyzed in all 52 patients using PCR with formalin-fixed, paraffin-embedded specimens. In each case, from 1 to 9 (mean, 2.7) samples in which a relatively dense lymphoid infiltrate was observed (a histological score of at least 2[7]) on H&E sections were subjected to PCR. Therefore, a total of 140 samples were studied by PCR. The specimens with a score of 1 (chronic active gastritis without lymphoid follicles) and those with a score of 0 (normal gastric mucosa) were not tested by PCR. The extraction of DNA was performed according to previously described methods.[14,24,25] Briefly, after 8-μm tissue sections cut from paraffin blocks were deparaffinized and dried, 100 μl of digestion buffer containing 200 μg/ml proteinase K (Wako Chemical Industries, Osaka, Japan), 50 mmol/L Tris/HCl (pH 8.5), 1 mmol/L EDTA, and 0.5% Tween 20 were added to each sample. After incubation overnight at 37°C, the samples were heated at 95°C for 9 minutes.

Semi-nested PCR was performed using primers FR3A and LJH for round 1 and FR3A and VLJH for round 2.[14,15] Briefly, a round 1 reaction contained 5 μl of template DNA extract, 20 pmol each of FR3A and LJH primers, 0.2 mmol/L of each dNTP, and 0.6 U of *Taq* polymerase (Perkin Elmer, Norwalk, CT) in the PCR buffer, with a total volume of 30 μl per reaction; for round 2, the same buffer was used except the VLJH primer was substituted for LJH and a 3-μl aliquot of round 1 was used as the template DNA. Amplifications were performed using a Gene Amp PCR System 9600 (Perkin Elmer). Both round 1 and round 2 consisted of 30 cycles of 94°C denaturation for 1 minute, 60°C annealing for 1 minute, and a 72°C extension for 1 minute. The products of round 2 PCR were electrophoresed in 10% polyacrylamide gels and visualized with silver staining according to the manufacturer's protocol (Daiichi Pure Chemicals, Tokyo, Japan).

Statistics

Values are expressed as mean ± SD unless stated otherwise. Statistical differences were evaluated using either the χ^2 test or the Mann-Whitney *U* test. A value of $P < 0.05$ for each test was regarded as statistically significant. The relative risk of B-cell monoclonality for gastric MALT lymphoma was determined by the odds ratio.[26]

Results

Comparison between Group A and Group B

A comparison of the histopathologic findings between groups A and B is shown in Table 2 and Figure 1. There was no difference between the two groups at the initial observations regarding the histological score for lymphoma (2.0 ± 1.0 for group A and 2.1 ± 1.1 for group B) or *H. pylori* density (1.8 ± 1.0 and 1.8 ± 0.8, respectively), in addition to the frequency of *H. pylori* positivity. At the final observations, however, significant differences were found regarding the histological score (4.5 ± 0.5 and 1.8 ± 0.8, $P < 0.001$) and *H. pylori* density (0.7 ± 0.9 and 1.9 ± 1.0, $P < 0.001$; Figures 2 and 3). The changes in *H. pylori* density (final minus initial density) per 1000 days was −1.4 ± 1.7 for group A and +0.2 ± 2.2 for group B ($P < 0.005$).

At the time when the histologically neoplastic specimens (scores for lymphoma were 4 or 5) were obtained in group A, the *H. pylori* density did not differ between 66 specimens taken from the endoscopically affected lesions (0.9 ± 1.0) and 48 specimens taken from the unaffected areas (0.9 ± 0.9).

B-cell monoclonality determined by the IgH gene rearrangement was frequently detected in group A (79%; Figure 2f), whereas it was seen in only 5 of the 24 patients (21%) in group B (Figure 4 and Table 2; $P < 0.005$). In these five patients, the histological scores for lymphoma of the specimens that showed monoclonality were all score 3, with no clear evidence of lymphoma being rec-

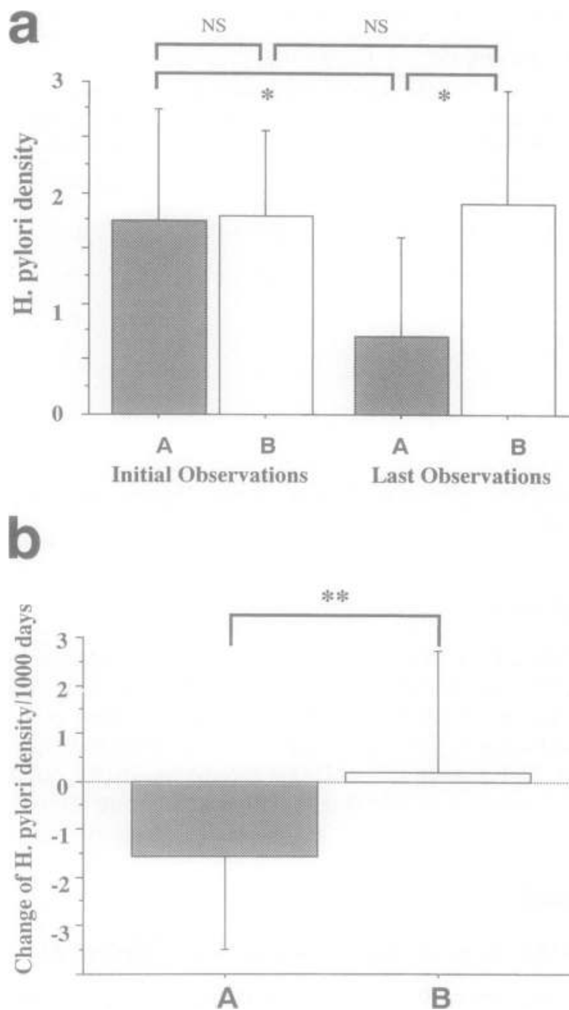

Figure 1. a: A comparison of *H. pylori* density at initial and final observations between group A and group B. **b:** A comparison of the changes in *H. pylori* density per 1000 days between group A and group B. NS, not significant. $^{*}P < 0.001$; $^{**}P < 0.005$ (Mann-Whitney *U* test).

ognized either clinically or histologically during the follow-up period (8 to 106 months after the determination of monoclonality). In addition, monoclonality was detected in only one of every five patient specimens, with all other subsequent specimens being polyclonal. In contrast, of the 22 patients in group A who showed monoclonality, monoclonality was detected in two or more of the repeated biopsy specimens in 14 patients (64%) but in only one specimen in 8 patients. Compared with the patients who showed no monoclonality, those showing monoclonality at least once in the subsequent specimens detected by PCR demonstrated an odds ratio for developing gastric MALT lymphoma of 13.9 (as calculated from Table 2).[26]

Comparison between the Neoplastic and Reactive Specimens

Based on the histological scores for MALT lymphoma, all examined specimens in both groups A and B were thus classified as either neoplastic (scores of 4 or 5) or reactive (scores up to 3). Among them, 140 specimens (48 neoplastic and 92 reactive) in which B-cell clonality was assessed by PCR were comparatively analyzed in detail, as shown in Table 3. Compared with reactive specimens, the neoplastic specimens showed a significantly lower degree of *H. pylori* density and neutrophilic activity, a higher degree of chronic inflammation, and a greater presence of lymphoepithelial lesions. Monoclonality was found not only in neoplastic (63%) but also in histologically reactive specimens (26 of 92, 28%); however, 21 of these 26 (81%) reactive specimens were obtained from group A patients who developed lymphoma during the follow-up.

Regarding the degree of *H. pylori* density and intestinal metaplasia, no correlation was found either when assessed in all 140 specimens (Spearman's $r = 0.12$, $P = 0.18$) or when assessed in only 92 histologically reactive specimens ($r = 0.08$, $P = 0.44$).

Comparison between the Monoclonal and Polyclonal Specimens

Table 4 summarizes the results of the histopathological comparison between 56 monoclonal specimens and 76 polyclonal specimens in both groups A and B, as determined by PCR results. The remaining eight specimens, which showed an equivocal, indeterminate pattern by PCR, were excluded. Monoclonal specimens showed a significantly higher lymphoma score and a higher degree of chronic inflammation than the polyclonal specimens. No differences were observed between the two groups based on clonality regarding the degree of *H. pylori* density, neutrophilic activity, and intestinal metaplasia or the presence of lymphoepithelial lesions and germinal centers. A strong correlation was also observed between monoclonality and group A ($P < 0.001$). Based on the values presented in Table 4, the accuracy of detecting B-cell clonality by PCR with a view to predicting the development of lymphoma (at that time and/or in the future) were as follows: a sensitivity of 57%, a specificity of 88%, a predictive value of a positive test of 91%, a predictive value of a negative test of 50%, and an efficiency of 67%.[27]

Time Lag between Histological and Molecular Genesis of Lymphoma

Of the 22 patients in group A in whom B-cell monoclonality was documented by PCR (Table 2), the dates when the neoplastic change was identified for the first time by histology (scores for lymphoma, 4 or 5) and by PCR were identical in only 6 (27%). In two patients (9%), monoclonality was detected only in specimens obtained after the malignant change had already been confirmed by histology. In many patients (14 of 22, 64%), the first detection of monoclonality by PCR preceded histological evidence of malignant transformation. Twenty-three specimens showing monoclonality taken from these 14

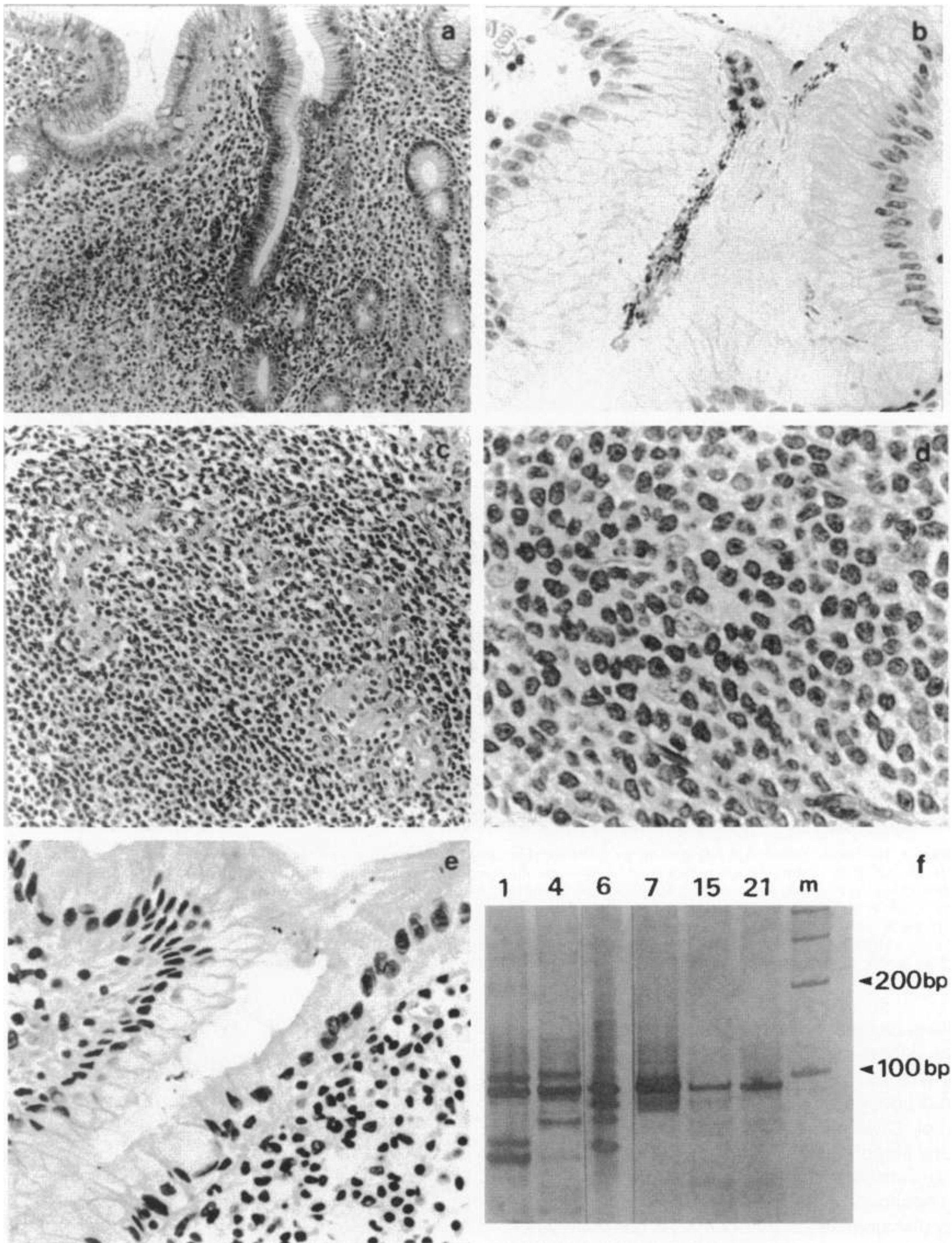

Figure 2. Group A. This male patient underwent total gastrectomy for gastric low-grade B-cell MALT lymphoma after a 122-month follow-up of *H. pylori*-associated chronic gastritis or reactive lymphoid hyperplasia. **a:** Initial biopsy specimen showing chronic active gastritis with lymphoid follicles (lymphoma score, 2; 54 years of age). H&E; magnification, ×138. **b:** Immunostaining for B471 of the initial biopsy specimen is shown. Many clusters of *H. pylori* bacteria can be seen in the mucosal pit (*H. pylori* density, 3). DAB with hematoxylin counterstain; magnification, ×430. **c:** The final (21st) biopsy specimen showing a dense lymphoid infiltrate with typical lymphoepithelial lesions (lymphoma score, 5; 64 years of age). H&E; magnification, ×190. **d:** A high-power view of **c** shows a diffuse proliferation of centrocyte-like cells. H&E; magnification, ×610. **e:** Immunostaining for B471 of the final biopsy specimen showing only a few *H. pylori* bacteria (*H. pylori* density, 1). DAB with hematoxylin counterstain; magnification, ×430. **f:** PCR products for the IgH gene of the serial biopsy specimens. **Lanes 1, 4,** and **6** (the 1st, 4th, and 6th biopsy specimens) show a polyclonal pattern, whereas **lanes 7, 15,** and **21** (the 7th, 15th, and 21st biopsy specimens) reveal a monoclonal rearrangement of the IgH gene. **Lane m**, size marker.

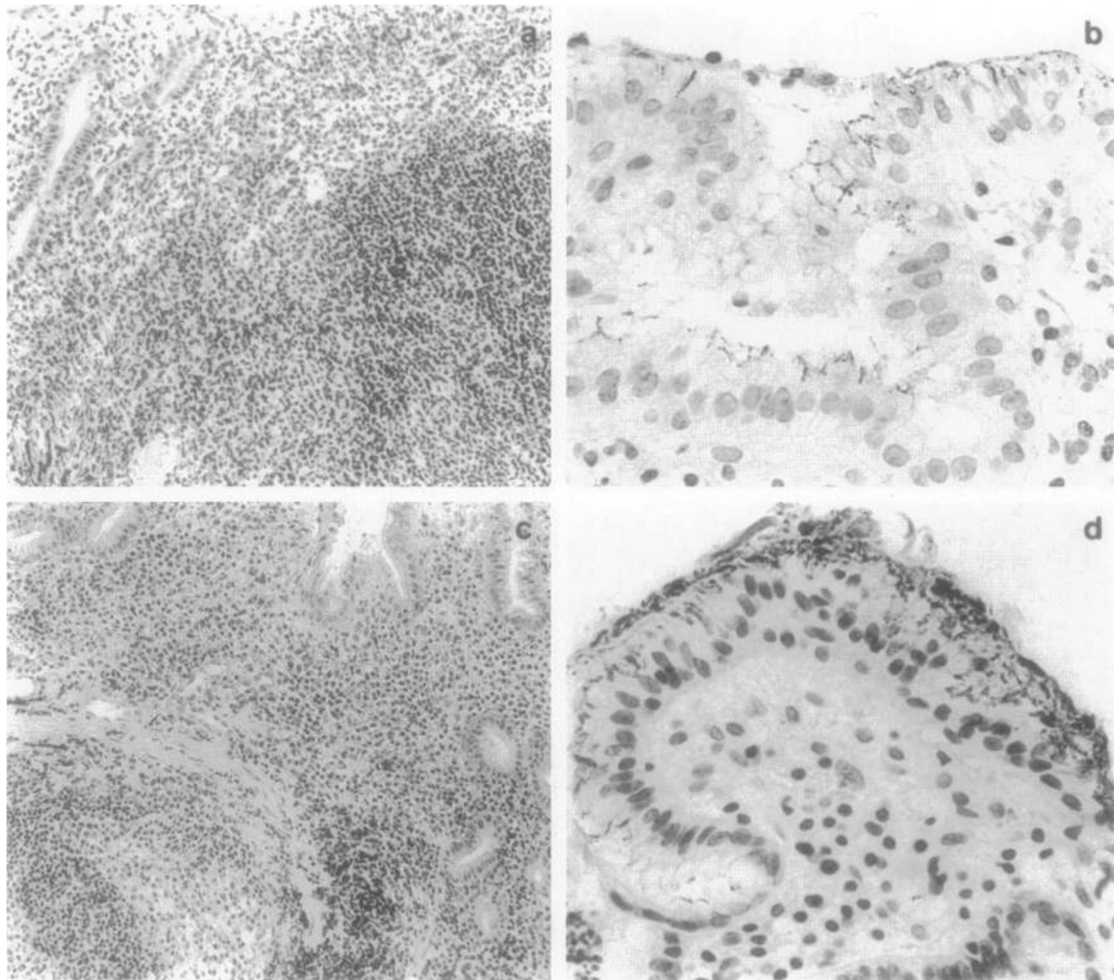

Figure 3. Group B. This female patient was followed up for 100 months because of reactive lymphoid hyperplasia (chronic gastritis) associated with *H. pylori*. PCR studies for the IgH gene of the subsequent biopsy and endoscopically resected specimens all showed a polyclonal pattern. a: The initial biopsy specimen showing a dense lymphoid infiltrate surrounding a lymphoid follicle and occasionally destroyed epithelial tubules (lymphoma score, 3; 49 years of age). H&E; magnification, ×160. b: Immunostaining for B471 of the initial biopsy specimen is shown. A moderate amount of *H. pylori* bacteria can be seen attached to the epithelium (*H. pylori* density, 2). DAB with hematoxylin counterstain; magnification, ×430. c: The final biopsy specimen shows chronic active gastritis with prominent lymphoid follicles. No lymphoepithelial lesions can be seen (lymphoma score, 2; 57 years of age). H&E; magnification, ×160. d: Immunostaining for B471 of the final biopsy specimen showing many clusters of *H. pylori* bacteria (*H. pylori* density, 3). DAB with hematoxylin counterstain; magnification, ×430.

patients were found to be histologically reactive; eighteen specimens demonstrated a score of 3, whereas five demonstrated a score of 2. Immunohistochemically, these monoclonal specimens occasionally showed small aggregates of CD20-positive lymphocytes,[15] but never showed any immunoglobulin light chain restriction, while also being indistinguishable from any other polyclonal reactive specimens. The time lag between histological and molecular genesis of lymphoma (ie, the date when the neoplastic changes were first detected by histology subtracted from the date when the neoplastic changes were first detected by PCR) in the 22 patients in group A who showed monoclonality was −380.4 ± 684.1 days.

Discussion

Although an etiological association between a *H. pylori* infection and the development of gastric MALT lymphoma has been suggested,[4–11,28–30] the role of *H. pylori*

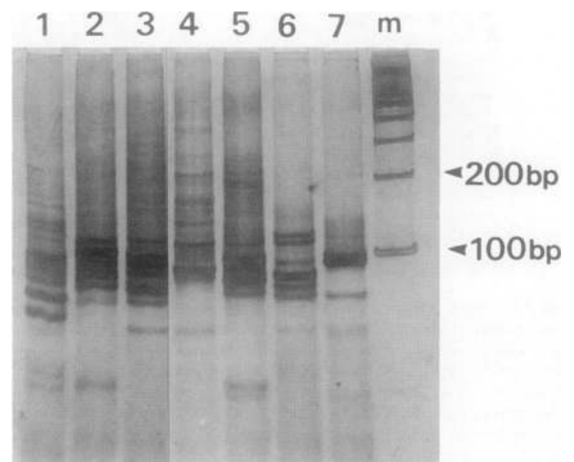

Figure 4. PCR products for the IgH gene in group B. Lanes 1 to 3, subsequent samples from the same patient as in Figure 3, show a polyclonal pattern. Lanes 4 to 7, samples from different patients; only lane 7 shows a monoclonal pattern, whereas all of the other lanes show polyclonal patterns.

Table 3. Comparison of the Histopthological and Molecular Findings between the Neoplastic and Reactive Specimens Determined by Histology

	H. pylori density	Neutrophilic activity	Chronic inflammation	Intestinal metaplasia	Lymphoepithelial lesions (*n* (%))	Germinal centers (*n* (%))	Patients (*n* (%))		B cell monoclonality		
							Group A	Group B	*n* (%)	Group A (*n*)	Group B (*n*)
Neoplastic (*n* = 48)	1.1 ± 1.0	0.7 ± 0.7	3.0 ± 0.1	0.4 ± 0.7	44 (92)	33 (69)	48 (100)	0 (0)	30 (63)	30	0
Reactive (*n* = 92)	1.6 ± 1.0*	1.0 ± 0.5†	2.5 ± 0.5†	0.8 ± 0.9‡	55 (60)§	66 (72)¶	48 (52)§	44 (48)	26 (28)§	21	5

*$P < 0.01$ by Mann-Whitney *U* test.
†$P < 0.001$ by Mann-Whitney *U* test.
‡$P = 0.066$ by Mann-Whitney *U* test.
§$P < 0.001$ by χ^2 test.
¶Difference not significant by χ^2 test.

in the pathogenesis of gastric lymphoma still remains unclear. To our knowledge, no previous studies have evaluated the temporal changes of *H. pylori* density and B-cell clonality that take place during the evolution from chronic gastritis to malignant lymphoma.

In the current study, we demonstrated that *H. pylori* density in group A decreased significantly during the long-term period, in comparison with that in group B (Figure 1). This finding suggests that *H. pylori* is associated more closely with the precursor or initial phase in the genesis of gastric MALT lymphoma than with the later phase, as its density decreases as the tumor develops or progresses further. Although this study is a retrospective one and the possibility of a sampling error due to the small biopsy specimens cannot be completely ruled out, we believe that our results are reliable because the biopsies were taken not only from affected lesions but also from the unaffected antrum and corpus in all patients, and the *H. pylori* density did not differ between the lesions and unaffected areas in group A.

The mechanisms behind the decrease in *H. pylori* in the genesis of lymphoma are uncertain. The destruction of mucosal tubules by lymphoma cells in wide areas may influence this decrease to some extent, this perhaps being partly supported by our previous studies showing that the size of gastric lymphoma was generally larger than that of gastric carcinoma[18,31] and that *H. pylori* density was lower in larger-sized lymphomas than in smaller ones.[11] In the patients with gastric carcinoma, a decrease in *H. pylori* in the stomach is considered to be caused by mucosal atrophy with intestinal metaplasia,[3,32–34] although the loss of *H. pylori* in the patients with gastric lymphoma seems to be independent of the progression of intestinal metaplasia, as in our study, *H. pylori* density did not correlate with the degree of intestinal metaplasia ($P = 0.17$ in 140 examined specimens). Moreover, the scores for intestinal metaplasia tended to be lower than those in the reactive specimens (Table 3). Conversely, a low degree of neutrophilic activity in the lymphoma specimens is more likely to be associated with a decrease in *H. pylori* (Table 3), as neutrophils are considered to be a sensitive indicator of the presence or absence of *H. pylori*.[22]

In a recent review of Isaacson,[35] he pointed out that the regressed low-grade MALT lymphomas following the eradication of *H. pylori* were all in an early phase, and he speculated that such cases with *H. pylori*-dependent low-grade MALT lymphoma may be induced by some genetic changes, such as trisomy 3,[36] in the patients with *H. pylori* infection and that further genetic changes such as t(1; 14)[37] or p53 abnormalities[38] could result in *H. pylori*-independent MALT lymphoma or else high-grade transformation.[35] Our results, which showed a decrease in *H. pylori* density during the genesis of lymphoma, thus seem to partly support the hypothesis of a transition from *H. pylori*-dependent MALT lymphoma to *H. pylori*-independent tumors, because such *H. pylori*-negative MALT lymphomas seem unlikely to regress after antibiotic therapy; however, this speculation awaits future confirmation from additional clinical trials.

The significance of the detection of B-cell monoclonality by PCR remains controversial, because monoclonality is detected not only in the neoplastic[7,9,10,12–14] but also in the histologically reactive lesions.[15,16] In a report by Savio et al,[10] 2 of 55 cases (4%) with gastric reactive lymphoid infiltrate showed a monoclonal PCR pattern,

Table 4. Comparison of the Histopathological Findings between the Monoclonal and Polyclonal Specimens Determined by PCR

	Lymphoma score	*H. pylori* density	Neutrophilic activity	Chronic inflammation	Intestinal metaplasia	Lymphoepithelial lesions (*n* (%))	Germinal centers (*n* (%))	Patients (*n* (%))	
								Group A	Group B
Monoclonal (*n* = 56)	3.6 ± 0.9	1.3 ± 1.1	0.9 ± 0.7	2.8 ± 0.4	0.5 ± 0.8	44 (79)	40 (71)	51 (91)	5 (9)
Polyclonal (*n* = 76)	2.8 ± 1.0*	1.5 ± 1.0†	0.9 ± 0.6†	2.5 ± 0.6*	0.8 ± 0.9‡	49 (64)§	53 (70)§	38 (50)¶	38 (50)

*$P < 0.001$ by Mann-Whitney *U* test.
†Differences not significant by Mann-Whitney *U* test.
‡$P = 0.113$ by Mann-Whitney U-test.
§Differences not significant by χ^2 test.
¶$P < 0.001$ by χ^2 test.

whereas Hsi et al[15] described how 6 of 41 cases (15%) with chronic active gastritis showed monoclonality. Moreover, Sorrentino et al[16] reported that 9 of 25 (36%) patients with follicular gastritis showed monoclonality. In our current study, monoclonality was detected not only in group A (79%) but also in group B (21%; Table 2). Therefore, a diagnosis of B-cell malignancy should not be based solely on the detection of a monoclonal rearrangement of the IgH gene by PCR from only one specimen.[15] However, when monoclonality is confirmed in repeated biopsies, we should consider the lesion to be either a lymphoma or prelymphomatous condition, as none of the patients in group B but 64% of the patients in group A showed monoclonality in two or more of their repeated biopsy specimens.

It is of interest that the first detection of monoclonality by PCR tended to precede the histological evidence of malignant change in group A (mean, −380.4 days). Neither histological nor immunohistochemical examinations could distinguish the specimens that were histologically reactive but genetically monoclonal from other reactive specimens. It thus seems reasonable that genetic monoclonality precedes cellular and/or histological neoplastic transformation,[16,39] although in previous studies this phenomenon has not been confirmed by follow-up observations. In addition to the very high odds ratio (13.9) calculated from Table 2, this precedence of B-cell monoclonality thus suggests the possible usefulness of the PCR technique for predicting the development of lymphoma in cases of relatively severe gastritis. High specificity (88%) and the predictive value of a positive test (91%) as calculated from Table 4 may also support the validity of this PCR test. In contrast, the sensitivity (57%), predictive value of a negative test (50%), and efficiency (67%) were all rather low; however, this may have been due to the use of paraffin-embedded tissue specimens rather than fresh-frozen materials.[12] Nevertheless, once monoclonality is detected by PCR, a follow-up biopsy for a subsequent PCR should be carried out to rule out any false positive cases. This PCR test was thus considered to provide valuable additional information for identifying those patients with a higher risk of developing lymphoma subsequent to relatively severe chronic gastritis. Additional investigations, including prospective studies of a large number of patients, are essential to fully clarify the significance of this molecular finding in the evolution of gastric MALT lymphoma from *H. pylori*-associated chronic gastritis.

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