Significance of CXCR3 expression in gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type for predicting responsiveness to *Helicobacter pylori* eradication

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Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a distinct low-grade lymphoma that often regresses upon *Helicobacter pylori* eradication. It was reported that the chemokine receptor CXCR3 is expressed not only on activated T cells, but also on MALT lymphoma cells, and that CXCR3-positive B lymphocytes migrate or home to the MALT of MALT lymphoma. In the present study, we aimed to elucidate the correlation between CXCR3 expression and the clinicopathological features of gastric MALT lymphoma, and to determine whether CXCR3 expression was predictive of responsiveness to *H. pylori* eradication. Sixty-seven patients with gastric MALT lymphoma in a single-center study were treated with *H. pylori* eradication therapy. We evaluated the correlation of CXCR3 expression with response to *H. pylori* eradication therapy by logistic regression stratified according to potential confounders. Immunohistochemical analysis revealed that 28 of 67 cases (42%) were positive for CXCR3 expression. CXCR3 expression was significantly more prevalent in those without *H. pylori* infection, advanced-stage disease, and in those with *API2–MALT1* fusion. In overall analysis, those with CXCR3 expression showed a significantly increased risk of non-responsiveness to *H. pylori* eradication therapy (odds ratio = 28.6; 95% confidence interval 5.70–143.4) compared to those without CXCR3 expression. This higher risk was observed consistently regardless of sex, *API2–MALT1* fusion, *H. pylori* infection, or clinical stage. We showed that CXCR3 expression was an independent predictive factor for non-responsiveness to *H. pylori* eradication therapy in patients with gastric MALT lymphoma. (*Cancer Sci* 2008; 99: 1769–1773)

Extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) is listed in the *World Health Organization Classification of Tumors of the Hematopoietic and Lymphoid Tissues* as a distinct low-grade lymphoma.[1] It develops in MALT acquired by chronic antigenic stimulation, and many of the cases occur in the stomach.[2,3] Research has demonstrated a strong association between chronic *Helicobacter pylori* infection and gastric MALT lymphoma;[4,5] therefore, *H. pylori* eradication has been recommended as the first-line therapy for this disease entity. Approximately 70% of early stage cases of gastric MALT lymphoma have been treated successfully with *H. pylori* eradication.[6–8]

Several studies have investigated possible predictive factors for responsiveness to *H. pylori* eradication as a therapeutic approach for gastric MALT lymphoma. We have identified the chromosomal translocation t(11;18)(q21;q21), which is seen exclusively in MALT lymphoma[9–12] and produces the *API2–MALT1* fusion gene product,[13–15] as an independent risk factor for non-responsiveness to *H. pylori* eradication.[16–19] Unfortunately, routine diagnostic detection of *API2–MALT1* via fluorescent in situ hybridization (FISH) or reverse transcription–polymerase chain reaction is not available for most patients, which limits the therapeutic application of this finding. Therefore, identification of markers other than the *API2–MALT1* fusion gene is essential for the routine prediction of *H. pylori* eradication responsiveness in gastric MALT lymphoma.

Chemokine receptors mediate the migration, activation, and proliferation of lymphocytes through the binding of specific ligands.[20] Jones *et al*. reported that the chemokine receptor CXCR3 is expressed not only on activated T cells, but also on MALT lymphoma cells.[21] Ohshima's group revealed the migration and homing behavior of CXCR3-positive B lymphocytes to the MALT of MALT lymphoma.[22,23] Taken together, these data suggest that CXCR3 expression may be associated with the clinical characteristics of MALT lymphoma, including responsiveness to treatment.

Here, we aimed to elucidate the clinicopathological features associated with CXCR3 expression in gastric MALT lymphoma and to further explore the value of CXCR3 for predicting *H. pylori* eradication responsiveness in gastric MALT lymphoma patients.

Patients and Methods

Patient samples. From October 1993 to December 2005, 67 patients with low-grade B-cell gastric MALT lymphoma were identified from the files of Aichi Cancer Center (Nagoya, Japan) and were enrolled in this single-center study. Cases that exhibited failure of *H. pylori* eradication or lacked sufficient clinical and follow-up data were precluded from this study. Informed consent was obtained from all patients with regard to the aims and protocol of the study.

Each patient had received *H. pylori* eradication therapy and follow-up analysis, and had been investigated regarding clinicopathological features and gene alterations at the Department of Gastroenterology, Aichi Cancer Center Hospital. The study population included 23 gastric MALT lymphoma patients who were the subjects of one of our previous reports.[16]

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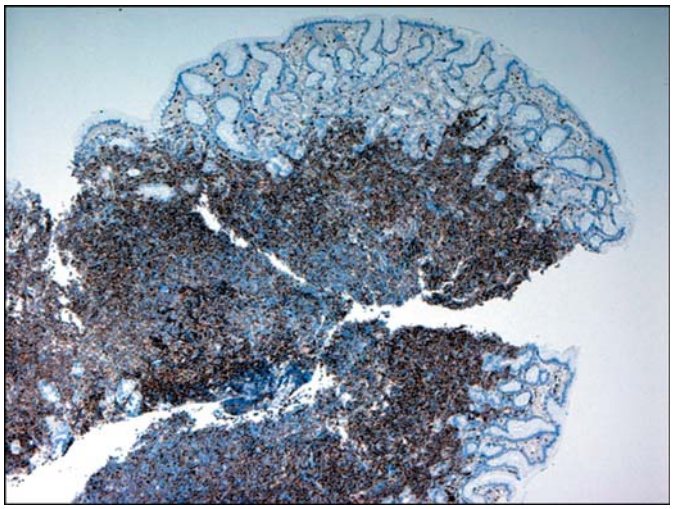

Fig. 1. Immunohistochemical staining. In gastric mucosa-associated lymphoid tissue lymphoma, CXCR3 was expressed in the cytoplasm and cell membrane of lymphoma cells.

Histological diagnosis, reverse transcription–polymerase chain reaction, and nucleotide sequencing studies for *API2–MALT1* chimeric transcripts. Formalin-fixed, paraffin-embedded lymphoma tissue obtained from endoscopic biopsy was processed for RNA isolation, and the histological diagnosis of gastric MALT lymphoma was reassessed according to the criteria of the World Health Organization classification by two pathologists at our hospital.[1] All samples were obtained and processed similarly. Gene aberration was analyzed via polymerase chain reaction according to a previously published method.[9,24] All 67 cases exhibited dense diffuse infiltration of centrocyte-like cells in the lamina propriae, with characteristic lymphoepithelial lesions. The presence of diffuse large-cell components was also evaluated. As defined by de Jong and colleagues, high-grade component was considered to be present when compact clusters or sheets of large atypical lymphoid cells (centroblast-like or lymphoblast-like cells) were observed in 1% or more of the neoplastic lymphoid population.[25] Immunophenotypic expression of pan-B-cell antigens, such as CD20 and CD79a, and lack of expression of CD5 and CD10 were confirmed in all cases. All of the samples were obtained from untreated patients at diagnosis before therapy.

Immunohistochemistry for CXCR3. We carried out immunohistochemical analysis on paraffin-embedded samples using anti-CXCR3 antibodies (Pharmingen, San Diego, CA, USA) according to a protocol published previously.[22,23] In the present study, we calculated the number of positively stained cells and categorized the specimens according to their number of positive lymphoma cells as follows: category 0, <10% of positive lymphoma cells; category 1, 10–29% of positive lymphoma cells; category 2, 30–49% of positive lymphoma cells; and category 3, >50% of positive lymphoma cells. The percentage of positive lymphoma cells was averaged to yield an immunohistological score of 0–100% by two independent pathologists. In the present study, no case was classified into category 1. Therefore, we defined a case as CXCR3-positive when more than 30% of the neoplastic B cells showed cytoplasmic CXCR3 expression (Fig. 1). This criterion was adopted to avoid overestimation because MALT lymphomas were often accompanied by varying numbers of reactive T cells with CXCR3 expression.

Clinical management of MALT patients. Disease was classified according to the Lugano staging system for gastrointestinal lymphomas, a modification of the Ann-Arbor classification system.[26]

Helicobacter pylori infection was detected by two or more of the following tests: histological evaluation; tissue culture; rapid urease test; urea breath test; and serological test for anti-*H. pylori* immunoglobulin G. Each case was judged as positive for *H. pylori* if at least one test was positive. After clinical staging, all 67 patients, both positive and negative for the infection, were treated for *H. pylori* eradication by a 2-week course of antibacterial treatment (clarithromysin, 200 mg twice daily; amoxicillin, 500 mg three times daily; and lansoprazole, 30 mg once daily). The effects of *H. pylori* eradication were evaluated 6 weeks after the treatment. When two or more tests were negative, *H. pylori* was considered eradicated. Follow up was carried out via upper gastrointestinal endoscopy; biopsy specimens were obtained from the same site as those taken during the pretreatment examination, and abdominal computed tomography was done every 3 months in the first year, every 4 months in the second year, and at intervals of at least 6 months in the third year and beyond. The tumors that responded to *H. pylori* eradication usually showed rapid endoscopic improvement that preceded histological improvement. Endoscopic examinations were further repeated until the lymphoma showed complete response (CR) or was judged as non-responsive. After achieving CR, patients were examined every 4–6 months. CR was judged when tumors showed CR both endoscopically and histologically. Those that failed to show histological regression 9 months after the successful eradication of *H. pylori* or that progressed during follow up were judged as non-responsive. Non-responders were subjected to alternative treatment strategies. Radiation therapy or surgery was recommended for patients with localized disease (stage I and II1), whereas chemotherapy was recommended for patients with advanced-stage disease (stage II2, IIE, and IV). The median follow-up period from *H. pylori* eradication was 31 months (mean 43 months, range 3–122 months). In addition to *H. pylori*-positive patients, *H. pylori*-negative patients were treated with the same eradication and follow-up protocols.

Statistical analysis. Correlation between the two groups was determined by means of χ^2 analysis, Fisher's exact test, and the Mann–Whitney *U*-test when appropriate. Univariate unconditional logistic regression models accompanied with stratification by potential confounders were applied to estimate the odds ratio (OR) and its 95% confidence interval (CI) for non-responsive to *H. pylori* eradication. Potential confounding variables considered in this analysis were sex (male vs female), *API2–MALT1* fusion (positive vs negative), *H. pylori* infection (yes vs no), and clinical stage (I vs II–IV). Statistical significance was set at $P < 0.05$. All statistical analyses were carried out using Statview 5.0 (SAS Institute, Cary, NC, USA).

Results

Clinicopathological features. The clinicopathological features of the CXCR3-positive ($n = 28$) and -negative ($n = 39$) patients are listed in Table 1.

There were no statistically significant differences in age, sex, or diffuse large-cell component between the two groups. The prevalence of *H. pylori* infection was higher in the CXCR3-negative group (97%) than in the CXCR3-positive group (71%). Stage I disease predominated in both groups; however, those with stage II and IV disease were more frequent in the CXCR3-positive group (22%) compared with the CXCR3-negative group (3%). The prevalence of the *API2–MALT1* fusion mutation was higher in the CXCR3-positive group (43%) compared with the CXCR3-negative group (0%).

Predictors of *H. pylori* eradication non-responsiveness. In order to identify predictive factors for eradication-responsive tumors and to select potential confounders to the association of CXCR3 expression with *H. pylori* eradication, we carried out univariate analysis for responders and non-responders to *H. pylori* eradication

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Table 1. Clinicopathological features according to CXCR3 expression

Clinicopathological feature	CXCR3 positive ($n = 28$) n (%)	CXCR3 negative ($n = 39$) n (%)	P-value
Age at diagnosis (years)			0.4877
Mean	56.9	59.1	
SD	13.6	12.3	
Sex			0.9524
Male	12 (43)	17 (44)	
Female	16 (57)	22 (56)	
Helicobacter pylori infection			0.003
Yes	20 (71)	38 (97)	
No	8 (29)	1 (3)	
Clinical stage			0.0183
I	22 (78)	38 (97)	
II + IV	6 (22)	1 (3)	
Diffuse large-cell component			0.1743
Yes	2 (7)	8 (21)	
No	26 (93)	31 (79)	
API2–MALT1 fusion			<0.0001
Positive	12 (43)	0 (0)	
Negative	16 (57)	39 (100)	

Table 2. Clinicopathological characteristics of gastric mucosa-associated lymphoid tissue lymphoma according to the responsiveness to *Helicobacter pylori* eradication therapy

Clinicopathalogical feature	Non-responsive ($n = 19$) n (%)	Responsive ($n = 48$) n (%)	P-value
Sex			0.039
Male	12 (63)	17 (35)	
Female	7 (37)	31 (65)	
Age at diagnosis (years)			0.53
Mean	58.9	57.9	
SD	13.5	12.7	
API2–MALT1 fusion			<0.0001
Positive	12 (63)	0 (0)	
Negative	7 (37)	48 (100)	
Helicobacter pylori infection			<0.0001
Yes	11 (58)	47 (98)	
No	8 (42)	1 (2)	
Clinical stage			0.002
I	13 (68)	47 (98)	
II + IV	6 (32)	1 (2)	
Diffuse large-cell component			0.525
Yes	2 (11)	8 (17)	
No	17 (89)	40 (83)	

therapy. We evaluated the following six factors: sex, age, *API2–MALT1* fusion, *H. pylori* infection, clinical stage, and diffuse large-cell component (Table 2).

Responders were predominantly women ($P = 0.039$), all negative for *API2–MALT1* fusion ($P < 0.0001$), had a higher *H. pylori* infection rate ($P < 0.0001$), and a lower clinical stage ($P = 0.0020$) in comparison to non-responders. However, we detected no statistically significant difference in the large-cell component between responders and non-responders ($P = 0.525$). Therefore, we selected sex, *API2–MALT1* fusion, *H. pylori* infection, and clinical stage as potential confounding variables.

Significance of CXCR3 expression for H. pylori eradication therapy. In order to characterize the significance of CXCR3 expression, we evaluated the correlation between CXCR3 expression and responsiveness to *H. pylori* eradication by clinicopathological factor-stratified analysis. Table 3 shows the resulting OR for non-responsiveness to *H. pylori* eradication therapy for gastric MALT lymphoma relative to the expression of CXCR3.

In the overall analysis, CXCR3 expression was associated with a significantly increased risk of non-responsiveness to eradication (OR = 28.6; 95% CI, 5.70–143.4) compared with those without CXCR3 expression. This trend was observed across all potential confounders, indicating that CXCR3 expression is an independent risk factor for non-responsiveness to eradication therapy.

Discussion

Our series is the first to elucidate the relationship between CXCR3 expression on lymphoma cells and the clinicopathological features of patients with gastric MALT lymphoma. We analyzed a large series in a single-center study, and showed that CXCR3 expression is a predictive factor, independent of the *API2–MALT1* fusion mutation, for non-responsiveness to *H. pylori* eradication therapy, implying that CXCR3-positive tumors are less sensitive to this treatment than are CXCR3-negative tumors. This finding supports the hypothesis that CXCR3 expression is associated with the clinical characteristics of gastric MALT lymphoma, including responsiveness to treatment.

Recent studies showed that CXCR3 is a chemokine receptor present on activated T cells and expressed on leukemic B lymphocytes in MALT lymphoma. Also, recent research has shown that CXCR3 expression correlates with the capability of neoplastic B cells to migrate to the circulation and different lymphoid organs.[22,23] However, few data on the relationship between clinical parameters and CXCR3 expression in patients with gastric MALT lymphoma are available.

Here, we showed that some clinicopathological factors, including clinical stage, differ significantly between patients with CXCR3-positive and CXCR3-negative disease. These findings suggest that CXCR3 expression is related to the mechanism of migration and mucosal homing in lymphoid tissue of MALT lymphoma, and either migration or a homing mechanism might be associated with the responsiveness to eradication therapy.

Of note, it is straightforward to carry out routine diagnostic analysis of CXCR3 expression levels in a clinical laboratory.

There are several methodological issues that merit consideration. This was a retrospective study; however, selection or information bias is unlikely because all clinicopathological information was available prior to the collection of information about the responsiveness to *H. pylori* eradication therapy. It could be advantageous that we

Table 3. Expression of CXCR3 and responsiveness to *Helicobacter pylori* eradication therapy stratified by potential confounders

Variables	CXCR3	Non-responsive	Responsive	Odds ratio	95% confidence interval	*P*-value
All patients ($n = 67$)						
	Positive	17	11	28.6	5.70–143.4	<0.0001
	Negative	2	37	1.00	Reference	
	Total	19	48			
Sex						
Male ($n = 29$)						
	Positive	10	2	37.5	4.51–311.5	<0.0001
	Negative	2	15	1.00	Reference	
	Total	12	17			
Female ($n = 38$)						
	Positive	7	9	Infinity		
	Negative	0	22	1.00	Reference	
	Total	7	31			
API2–MALT1 fusion						
Positive ($n = 12$)						
	Positive	12	0	Infinity		
	Negative	0	0	1.00	Reference	
	Total	12	0			
Negative ($n = 55$)						
	Positive	5	11	8.41	1.43–49.5	0.008
	Negative	2	37	1.00	Reference	
	Total	7	48			
Helicobacter pylori infection						
Yes ($n = 58$)						
	Positive	9	11	14.7	2.76–78.6	<0.0001
	Negative	2	36	1.00	Reference	
	Total	11	47			
No ($n = 9$)						
	Positive	8	0	Infinity		
	Negative	0	1	1.00	Reference	
	Total	8	1			
Clinical stage						
I ($n = 60$)						
	Positive	12	10	44.4	5.14–383.6	<0.0001
	Negative	1	37	1.00	Reference	
	Total	13	47			
II + IV ($n = 7$)						
	Positive	5	1	0		
	Negative	1	0	1.00	Reference	
	Total	6	1			

studied a relatively large series in a single-center study; however, our results need confirmation from studies carried out at other centers and in other populations.

In summary, our data show that CXCR3 expression is significantly associated with the clinicopathological features of gastric MALT lymphoma, and is a predictive factor, independent of the *API2–MALT1* fusion mutation, for non-responsiveness to *H. pylori* eradication therapy. Further studies examining the molecular mechanism of CXCR3 activity in gastric MALT lymphoma are warranted.

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