Helicobacter pylori gastritis and primary gastric non-Hodgkin's lymphomas

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Abstract

Aims—To evaluate further the relation between gastric malignant lymphoma of the mucosa associated lymphoid tissue (MALT) and *Helicobacter pylori*.

Methods—One hundred and sixty two surgical specimens of MALT lymphoma were retrospectively investigated to determine tumour type and inflammatory patterns. In 121 cases biopsy specimens obtained before surgery were available and stained with haematoxylin and eosin, periodic acid Schiff, Giemsa and Warthin-Starry stains.

Results—Residual lymphoid follicles were found less often in high grade malignant than in low grade malignant MALT lymphomas. Chronic active gastritis was shown within the mucosa at some distance from the tumours in 143 of 146 specimens. In all the cases for which biopsy specimens could be evaluated, colonisation of the mucosa by *H pylori* had occurred. Lymphoid follicles and lymphoid aggregates were detected in 82.7% of the antral, and in 85% of the body mucosa specimens.

Conclusions—These data support the hypothesis that *H pylori* has an important role in the development of MALT lymphomas. Furthermore, the chronic inflammation preceding malignant transformation might enhance the probability of malignant transformation via chronic stimulation of the lymphoid tissue. This might in part indicate why MALT lymphomas occur most often in the stomach.

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One of the most exciting current developments in gastroenterology is the discovery that *Helicobacter pylori* has a decisive role in gastroduodenal disease.¹⁻³ Recently, several groups have published data showing a correlation between *H pylori* and gastric carcinomas.⁴⁻⁶ Wotherspoon *et al* were the first to investigate the presence of *H pylori* in larger numbers of gastric lymphomas of the mucosa associated lymphoid tissue (MALT lymphomas): in 92% of cases *H pylori* was indeed detected.⁷ On the basis of earlier observations that the prevalence of lymphoid follicles correlated significantly with the detection of *H pylori*,^{8,9} they suggested that *H pylori* might trigger the acquisition of MALT in the gastric mucosa which usually contains only a few T lymphocytes.¹⁰ In the light of these findings *H pylori*

and the subsequent inflammatory response might be prerequisites for the development of a MALT lymphoma in the stomach. Further support for this hypothesis was provided by data published by Doglioni and coworkers which showed that the high prevalence of gastric MALT lymphoma correlated closely with a high prevalence of *H pylori* infection in a town in northern Italy.¹¹ If the suggested route to MALT through *H pylori* infection to the malignant transformation holds true, then not only *H pylori* but an inflammatory response to *H pylori* infection should be evident in almost all cases of MALT lymphoma as well. To elucidate further this association we retrospectively investigated 162 cases of gastric resection specimens for gastric lymphoma.

Methods

One hundred and sixty six surgical specimens of gastric lymphoma (56 gastrectomies, 95 arboral resections, nine local excisions, six resections of the gastric remnant) were examined at the Institute of Pathology, Bayreuth, over a period of 11 years. They were fixed in 10% formalin, embedded in paraffin wax and routinely stained with haematoxylin and eosin, periodic acid Schiff and Giemsa. The lymphomas were classified according to the proposal of the European Lymphoma Study Group.¹² Only B cell lymphomas of MALT type were included in this study. Two plasmacytomas and two T cell lymphomas were excluded, reducing the number of cases investigated to 162.

The phenotypic evaluation of lymphomas was done using monoclonal antibodies recognising formalin fixation resistant antigens (L26, UCHL1, MT1, all from Dako) and a subsequent peroxidase-antiperoxidase method. The high grade malignant MALT lymphomas were divided into those with and without a low grade component. Furthermore, the depth of infiltration into the gastric wall was evaluated using the International Union against Cancer classification of gastric cancer (table 1).¹³ Clinical staging of lymphoma was also carried out in all patients using abdominal computed tomograms or ultrasonography, liver and bone marrow biopsies

Table 1 UICC classification of gastric carcinoma

pT1	Tumour restricted to mucosa and submucosa
pT2	Tumour infiltrating beyond the submucosa into the muscularis propria and the subserosal layer
pT3	Tumour penetrating the serosa
pT4	Tumour continuously infiltrating other organs (liver, spleen)

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Table 2 Modified Ann Arbor classification for extranodal lymphomas

E I	Lymphoma restricted to gastrointestinal tract on one side of the diaphragm
E I ₁	Infiltration limited to mucosa and submucosa
E I ₂	Lymphoma extending beyond submucosa
E II	Lymphoma additionally infiltrating lymph nodes on the same side of diaphragm
E II ₁	Infiltration of regional lymph nodes
E II ₂	Infiltration of lymph nodes beyond regional
E III	Lymphoma infiltrating gastrointestinal tract and/or lymph nodes on both sides of the diaphragm
E IV	Localised infiltration of gastrointestinal site with or without infiltration of associated lymph nodes together with diffuse or disseminated involvement of extra-gastrointestinal organs.

with subsequent classification according to the modified Ann Arbor scheme^{14,15} (table 2), and only tumours in which the largest tumour mass was located in the stomach. The presence of non-neoplastic reactive lymphoid follicles within the tumours was registered.

In 146 cases it was also possible to evaluate the gastric mucosa at least 4 cm away from the tumours for infiltration of the lamina propria with lymphocytes, plasma cells, and neutrophilic polymorphs as well as intestinal metaplasia. The prevalence of lymphoid aggregates and follicles was also registered. The latter were diagnosed when germinal centres could be detected. *H. pylori* gastritis was diagnosed when a lymphoplasmacellular infiltrate was combined with neutrophilic polymorphs concentrated around the neck region with infiltration of gastric glands. In accordance with these criteria, all cases of chronic active gastritis were classified as *H. pylori* gastritis. Because of the influence of delayed fixation on the detection of *H. pylori* (M Stolte, unpublished observations) we also investigated endoscopic biopsy specimens taken three months or less before the surgical procedure for colonisation of the gastric mucosa with *H. pylori*; such biopsy specimens were available in 121 cases. *H. pylori* was detected using the Warthin-Starry stain. Lymphocytic and reactive gastritis were diagnosed according to the criteria given before.^{16,17}

Statistical evaluation was done using the χ^2 test.

Results

The mean age of the patients (74 men, 88 women) was 62.2 years, with a standard deviation of 13.1 years and a range from 20 to 92 years. The types of lymphomas detected, together with their prevalences, are shown in table 3. The staging of the tumours according to the Ann Arbor classification showed that most were only locally infiltrating: E I₁ in 72 (44.4%) cases and E I₂ in 33 (20.4%) cases. Only in 41 (25.3%) patients was an infiltration of regional lymph nodes detected (E II₁),

Table 3 Histological classification of gastric lymphomas (n = 162) and their prevalence (MALT mucosa associated lymphoid tissue)

Low grade malignant MALT lymphomas	91
High grade malignant MALT lymphomas with low grade component	21
without low grade component	50

Table 4 Breakdown of depth of tumorous infiltration of gastric wall by degree of malignancy (n = 162)

	Low	Low + high	High
pT1	66 (74.2%)	10 (11.2%)	13 (14.6%)
pT2	18 (45.0%)	6 (15.0%)	16 (40.0%)
pT3	6 (22.2%)	5 (18.5%)	16 (59.3%)
pT4	1 (16.7%)		5 (83.3%)

p < 0.001.

and in four (2.5%) cases had infiltrated distant lymph nodes. Liver or spleen metastases were found in three (1.9%) cases and bone marrow infiltration in two (1.2%). In seven (4.3%) patients in whom only a local excision was performed no classification was possible. The correlation between the depth of infiltration and the grade of the lymphomas (table 4) reached significance (p < 0.001). The largest tumour diameters are shown in table 5. The types of gastritis detected in the mucosa located at a distance from the tumours are listed in table 6. In all the cases of chronic active gastritis the biopsy specimens taken before surgery showed *H. pylori* colonisation of the gastric mucosa. A correlation between largest diameters, grade, or depth of infiltration could not be demonstrated. Reactive non-neoplastic lymphoid follicles were found within the tumours in 62.0% of cases. An analysis of their correlation with degree of malignancy and with depth of infiltration reached significance in the case of the former (p < 0.001) (table 7). In the latter, however, owing to the small number of cases in the pT4 group, statistical analysis was possible only by pooling pT3 and pT4 lesions (p < 0.05) (table 8). The prevalence of lymphoid follicles and lymphoid aggregates in the antral and

Table 5 Largest tumour diameters (n = 147)

≤ 20 mm	<40	<60	<80	>80
22 (15.0%)	41 (27.9%)	37 (25.1%)	25 (17.0%)	22 (15.0%)

Table 6 Types of gastritis in gastric lymphomas of MALT (n = 162)

Insufficient material	16
Chronic active gastritis	143 (97.9%)
Lymphocytic gastritis	1 (0.7%)
Reactive gastritis	1 (0.7%)
No gastritis	1 (0.7%)

Table 7 Prevalence of lymphoid follicles (LF) within gastric lymphomas broken down by degree of malignancy

Low	70 (78.7%)
Low + high	13 (61.9%)
High	12 (24.0%)

p < 0.001.

Table 8 Prevalence of lymphoid follicles (LF) within gastric lymphomas broken down by depth of malignant infiltration

pT1	60 (69.0%)
pT2	20 (51.3%)
pT3	12 (44.4%)
pT4	2 (33.3%)

p < 0.05 (pT3 together with pT4).

Table 9 Prevalence of lymphoid follicles (LF), lymphoid aggregates (LA) and their combination (LF/LA) in gastric mucosa away from the lymphomas

	LA+	LF+	LF/LA+	Total
Antral mucosa (n = 139)	49	48	18	115 (82.7%)
Oxyntic mucosa (n = 140)	60	43	16	119 (85.0%)

Table 10 Prevalence of intestinal metaplasia (IM) in gastric mucosa at a distance from the lymphomas

Antral mucosa (n = 139)	54 (38.8%)
Oxyntic mucosa (n = 140)	24 (17.1%)

body mucosa is shown in table 9. The distribution of intestinal metaplasia of the gastric mucosa located at a distance from the lymphomas is shown in table 10. Significant correlations with the degree of malignancy or the depth of infiltration could not be shown.

Discussion

A MALT system as exemplified in Peyer's patches is not present in the normal gastric mucosa.¹⁰ Following infection with *H pylori*, MALT is acquired.⁷⁻⁹ Further evidence for this sequence of events is provided by the results of eradication studies. After successful eradication of *H pylori*, the density of the lymphoplasmacellular infiltration in the lamina propria decreased substantially¹⁸ and lymphoid aggregates and lymphoid follicles, present before were no longer detectable in control biopsy specimens (unpublished own observations). More support for the hypothesis that *H pylori* plays an important part in the genesis of MALT lymphoma comes from recent serological, epidemiological, and histopathological research. Parsonett *et al* reported in 1991 that in patients with gastric lymphomas antibodies against *H pylori* were present in 90.9% compared with 63.6% of matched controls.⁶ They only investigated 11 patients. Wotherspoon *et al* histologically evaluated 110 MALT lymphomas of the stomach in both biopsy and surgical specimens, looking for the presence of *H pylori*, and found the organisms in 92% of cases.⁷ This prevalence of *H pylori* was significantly higher than that reported for non-ulcer dyspepsia (50–60%).¹⁹ An analysis of epidemiological data published by Doglioni *et al* showed a high prevalence of primary gastric lymphoma in a town in northern Italy, correlated with a high prevalence of *H pylori* infection. Similarly, a low prevalence of gastric lymphoma was observed in a number of British towns with a low prevalence of *H pylori*.¹¹

The data presented here are based exclusively on findings in surgical specimens, and for the first time the gastritis associated with the lymphomas has been investigated as well. An extremely high prevalence of chronic active gastritis with features typical of *H pylori* aetiology was demonstrated in gastric MALT lymphoma (97.7%), compared with the results of a study of 5000 gastric biopsy specimens of various forms of gastritis at our insti-

tution. Only 71.8% of cases showed *H pylori* gastritis.²⁰ The results of epidemiological analyses of the prevalence of HP in a control population indicated a prevalence ranging from 61% to 76%.⁵⁻⁶ Biopsy specimens taken before surgery confirmed the assumed *H pylori* aetiology in our cases of chronic active gastritis. In addition, one case each of lymphocytic and reactive gastritis were found (table 6). Lymphocytic gastritis is mostly triggered by *H pylori* infection and for the most part represents a special type of reaction to *H pylori* so this case might be included in the category of gastritis induced by *H pylori*.²¹ Almost all MALT lymphomas therefore, show an inflammatory infiltrate in the gastric mucosa most probably caused by *H pylori*.

Another point hinting at the importance of *H pylori* is the enormous prevalence of lymphoid follicles and lymphoid aggregates and intestinal metaplasia found in the gastric mucosa at a distance from the tumour (tables 9 and 10). Comparing these data with the published prevalences for benign gastroduodenal disease, in particular in the body mucosa, a substantial increase in lymphoid aggregates, lymphoid follicles, and intestinal metaplasia was noted in MALT lymphoma.²²⁻²³ Intestinal metaplasia has been shown to be more prevalent in older patients, and probably represents longstanding *H pylori* gastritis.²³⁻²⁴ All in all, an analysis of the gastric mucosa showed, in almost all cases of MALT lymphoma, evidence for longstanding HP gastritis with acquisition of MALT. Our data thus support the hypothesis of Wotherspoon *et al* that *H pylori* has a crucial role in the evolution of gastric lymphomas of the MALT type by triggering the acquisition of MALT in the stomach mucosa.⁷ The two cases of MALT lymphomas in which no *H pylori* induced inflammatory reaction was detected do pose a problem in that respect. It is, however, possible to speculate that in these cases *H pylori* had been eradicated after a malignant transformation of the acquired MALT.

It might be argued, however, that *H pylori* gastritis develops secondary to MALT lymphoma. In contrast to patients with chronic gastritis, in whom the prevalence of *H pylori* increases with increasing age, we found no increased prevalence of *H pylori* in older patients.²⁵ Furthermore, no correlation of the *H pylori* colonisation with depth of infiltration, grade, or largest diameters of the lymphomas was to be found—as ought to be the case in secondary colonisation of MALT lymphomas.

With respect to the malignant transformation of MALT to MALT lymphoma, apart from the relatively safe assumption that MALT lymphomas develop from para-follicular centrocytoid-like cells of the para-follicular region,²⁶ the exact mechanisms are unknown. The continuing stimulation of the local immune system following *H pylori* infection might enhance the probability of its malignant transformation, especially as the infection persists throughout the patient's life if left to its natural course, as can be deduced from age distribution prevalence.²⁵ That chronic

infection can trigger lymphoproliferation has been shown in Epstein-Barr infection.²⁷⁻²⁸ If the assumption is correct that *H. pylori* infection is not merely the basis for, but actively contributes to, the aetiology of gastric lymphoma, then in view of the far greater prevalence of *H. pylori* infection than of MALT lymphoma, additional factors must operate. These might include peculiarities of the *H. pylori* strain involved: the high prevalence of lymphoid follicles and lymphoid aggregates might be interpreted as features of a possible high immunogenicity. Furthermore, exogenic—for example, dietary factors, virus infections, or disorders of immune regulation—have to be taken into consideration. The chronic inflammation caused by *H. pylori* could be an important part of the answer to the question why the stomach has the highest prevalence of MALT lymphomas,²⁹⁻³¹ although in the normal stomach only a few lymphocytes are found in the lamina propria and epithelium.¹⁰ Studies aimed at analysing the additional factors mentioned and estimating the possible benefit of eradication of *H. pylori* are in progress.

Although many questions concerning MALT lymphomas remain to be elucidated, we conclude that our results, together with the reported data, strongly support the hypothesis that *H. pylori* gastritis, by leading to the acquisition of MALT, is a prerequisite for the development of a MALT lymphoma in the stomach in almost all cases. Whether *H. pylori* and the inflammatory response also actively contribute to the formation of lymphomas in the stomach has yet to be determined.

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- 1 Marshall BJ, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, *et al.* Prospective double blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. *Lancet* 1988;330:1437-42.
- 2 Fiocca L, Solcia E, Santoro B. Duodenal ulcer relapse after eradication of *Helicobacter pylori*. *Lancet* 1991;337:1614.
- 3 Graham DY, Lew GM, Kleine PD, Evans DG, Evans DJ, Saeed ZA, *et al.* Results of treatment of *Helicobacter pylori* infection on the recurrence of gastric or duodenal ulcers: A randomized single-blind, single center study. *Gastroenterology* 1991;100:A 431.
- 4 Forman D, Newell DG, Fullerton F, Yarnell JWG, Stacey AR, Wald N. *et al.* Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *Br Med J* 1991;302:1302-5.
- 5 Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese-Americans in Hawaii. *N Engl J Med* 1991;325:1132-6.
- 6 Parsonett J, Vandersteen D, Goates J, Sibley RK, Pritikin J, Chang Y. *Helicobacter pylori* infection in intestinal- and diffuse-type gastric adenocarcinoma. *JNCI* 1991;83:640-3.
- 7 Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991;338:1175-6.
- 8 Wyatt JJ, Rathbone BJ. Immune response of the gastric mucosa to *Campylobacter pylori*. *Scand J Gastroenterol* 1988;23(suppl 142):44-9.
- 9 Stolte M, Eidt S. Lymphoid follicles of the antral mucosa: immune reaction to *Campylobacter pylori*? *J Clin Pathol* 1989;42:1269-71.
- 10 Kirchner T, Melber A, Fischbach W, Heilmann KL, Müller-Hermelink HK. Immunohistochemical patterns of the local immune response in *Helicobacter pylori* gastritis. In: Malfertheimer P, Dischuneit H, eds. *Helicobacter pylori, gastritis and peptic ulcer*. Berlin: Springer, 1990:213-22.
- 11 Doglioni C, Wotherspoon AC, Moschini A, De Boni M, Isaacson PG. High incidence of primary gastric lymphoma in north eastern Italy. *Lancet* 1992;339:834-5.
- 12 Isaacson PG, Spencer J, Wright DH. Classifying primary gut lymphomas. *Lancet* 1988;330:1148-9.
- 13 Hermanek P, Sobin LH eds. *TNM-Klassifikation maligner Tumoren*. 4th edition. Berlin: Springer, 1987.
- 14 Musshoff K, Schmidt-Vollmer H. Prognosis of non-Hodgkin's lymphomas with special emphasis on staging classification. *Z Krebsforschung* 1975;83:323-8.
- 15 Radaskiewicz Th, Dragosics B, Bauer P. Gastrointestinal malignant lymphomas of the mucosa-associated lymphoid tissue: factors relevant to prognosis. *Gastroenterology* 1992;102:1628-38.
- 16 Haot J, Hamuchi L, Walley L, Mainguet P. Lymphocytic gastritis: a newly described entity. A retrospective endoscopic and histological study. *Gut* 1988;29:1258-64.
- 17 Dixon MF, O'Connor HJ, Axon ATR, King RFJ, Johnston D. Reflux gastritis: a distinct histopathological entity? *J Clin Pathol* 1986;39:524-30.
- 18 Stolte M. *Helicobacter pylori* gastritis and gastric MALT-lymphoma. *Lancet* 1992;339:745-6.
- 19 Shallcross TM, Rathbone BJ, Heatley RG. *Campylobacter pylori* and non-ulcer dyspepsia. In: Rathbone BJ, Heatley RV, eds. *Campylobacter pylori and gastroduodenal disease*. Oxford: Blackwell Scientific Publications, 1989:155-6.
- 20 Stolte M, Bethke B, Ritter M, Lauer E, Eidt H. Praxis der Gastritis-Klassifikation. *Endoskopie heute* 1990;4:228-30.
- 21 Crabtree JE, Mahony MJ, Taylor JD, Heatley RV, Littlewood JM, Tompkins DS. Immune responses to *Helicobacter pylori* in children with recurrent abdominal pain. *J Clin Pathol* 1991;44:768-71.
- 22 Eidt S, Stolte M. Frequency distribution of lymphoid follicles and lymphoid aggregates in *Helicobacter pylori* gastritis in antral and body mucosa of the stomach. *Irish J Med Sci* 1992;161(suppl 10):A14.
- 23 Eidt S, Stolte M. Antral intestinal metaplasia in *Helicobacter pylori* gastritis. *Digestion* 1994;55:13-18.
- 24 Craanen ME, Decker W, Blok P, Ferweda J, Tytgat GNJ. Intestinal metaplasia and *Helicobacter pylori*: an endoscopic-biopsy study of the gastric antrum. *Gut* 1992;33:6-10.
- 25 Stolte M, Eidt S, Ohnsmann A. Differences in the *Helicobacter pylori* associated gastritis in the antrum and body of the stomach. *Z Gastroenterol* 1990;28:229-33.
- 26 Isaacson PG, Wotherspoon AC, Diss T, Pan L. Follicular colonization in B-Cell lymphoma of mucosa-associated lymphoid tissue. *Am J Surg Pathol* 1991;15:819-28.
- 27 Krueger GRF, Papadakis Th, Schaefer HJ. Persistent active Epstein-Barr virus infection and atypical lymphoproliferation. *Am J Surg Pathol* 1987;11:972-81.
- 28 Puertillo DT, Tatsumi E, Manolov G, Manolova Y, Harada S, Lipscomb H, *et al.* Epstein-Barr virus as an etiological agent in the pathogenesis of lymphoproliferative and aroliferative diseases in immune deficient patients. *Int Rev Exp Pathol* 1985;27:114-82.
- 29 Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. *Cancer* 1972;29:252-60.
- 30 Herrman R, Panahon AM, Barcos MP, Walsh D, Stutzman L. Gastrointestinal involvement in non-Hodgkin's lymphomas. *Cancer* 1980;46:215-22.
- 31 Otter R, Bieger R, Kluin PhM, Hermans J, Willemze R. Primary gastrointestinal lymphoma in a population-based registry. *Br J Cancer* 1989;60:745-50.