CASE REPORT

The natural history of a gastric low grade B cell MALT lymphoma followed during 11 years without treatment

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Low grade B cell mucosa associated lymphoid tissue (MALT) lymphoma of the stomach is usually an indolent tumour that remains localised for a long time before dissemination occurs. MALT appears in the stomach in response to infection by *Helicobacter pylori*, which is present in 80–90% of cases. The pathogenesis of the evolution from chronic gastritis to malignant lymphoma has not yet been fully explained and the exact role of *H pylori* in the pathogenesis and progression of gastric lymphoma remains unclear. This report describes the case of a 72 year old woman with a low grade B cell MALT lymphoma localised in the gastric fundus, who refused to be treated for eradication of *H pylori*. The histological diagnosis of B cell MALT lymphoma was supported by both immunohistochemical and molecular genetic analysis. After 11 years of follow up, this MALT lymphoma remained indolent, without local progression or blastic transformation, and the *H pylori* infection was still persistent, even though the density of bacteria had decreased drastically. Interestingly, two different clonal immunoglobulin (Ig) gene rearrangements were found in two series of biopsies performed with an interval of 11 years. This case report supports the following notions: (1) *H pylori* associated gastritis is a risk factor for gastric MALT lymphoma, but might not be sufficient by itself for the progression of the disease, and (2) in the evolution of MALT lymphomas, different cell clones characterised by different Ig rearrangements may emerge.

There is strong evidence that *Helicobacter pylori* infection plays a crucial role in the pathogenesis of gastric mucosa associated lymphoid tissue (MALT) lymphoma.^{1,2} This concept has been supported by the histological detection of *H pylori* in almost all gastric MALT-type lymphomas.^{1,3} However, it is not known whether the bacterium alone causes the evolution from chronic gastritis to MALT lymphoma or whether other pathogenic factors are involved. We previously showed in a series of gastric MALT lymphomas that *H pylori* infection is correlated with the grade and depth of invasion of MALT lymphoma and suggested that *H pylori* may play a promoter role in the development of MALT lymphoma, but that the progression of the lymphoma is not *H pylori* dependent.⁴

In this report, we describe the case of a 72 year old woman who presented with a B cell MALT lymphoma localised in the fundus for a period of 11 years. During this period, no treatment directed against *H pylori* had been administered and the lymphoma remained stable, indolent, and without either progression or blastic transformation. This case report supports the hypothesis that *H pylori* probably plays only a minor role in the progression of MALT lymphoma.

"It is not known whether the *Helicobacter pylori* alone causes the evolution from chronic gastritis to mucosa associated lymphoid tissue lymphoma or whether other pathogenic factors are involved"

CASE REPORT

A 72 year old woman with no particular medical history presented with a 10 month history of intermittent epigastric discomfort without vomiting, anorexia, or weight loss. An initial gastroscopy was performed and biopsy specimens of the antrum and fundus of the stomach showed a non-active mild chronic gastritis associated with *H pylori* infection. One year later endoscopy was repeated because of exacerbation of epigastric discomfort, and showed the fundic mucosae to be slightly thickened, suggesting a gastric lymphoma. A low grade B cell MALT lymphoma of the fundus was diagnosed. The patient refused to be treated by antibiotics to eradicate *H pylori* at that time. During the following 11 years, the patient continued to present with intermittent epigastric pain without other symptoms, but did not receive further treatment. Because of exacerbation of the epigastric pain, the patient was re-examined. Upon gastroscopy a limited region of the fundus showed thickening of the mucosae and biopsies were taken. Histologically the B cell MALT lymphoma had persisted and remained restricted to the gastric mucosa.

MATERIAL AND METHODS

Biopsy specimens had been fixed in formalin, embedded in paraffin wax, cut at 4 µm, and stained with haematoxylin and eosin. All histological material was reviewed. The presence of *H pylori* was determined in all gastric biopsies by means of a modified Giemsa stain.

Immunohistochemistry was performed on the three series of biopsies. Sections were stained by the PAP immunoperoxidase technique when using monoclonal antibodies L26 (dilution, 1/200) and pancytokeratin MNF116 (dilution, 1/50), and by the ABC technique for monoclonal antibody CD5 (dilution, 1/25) and the polyclonal anti-immunoglobulin (Ig) κ (dilution, 1/8000) and λ light chain (dilution, 1/2000) antibodies. Antibodies to Ig components, L26, and MNF116 were obtained from Dako (Glostrup, Denmark) and the antibody to CD5 was obtained from Novocastra (Newcastle, UK).

Analysis of Ig heavy chain gene rearrangement by the polymerase chain reaction (PCR) was performed on the three series of biopsies. Genomic DNA was extracted from 20–50 mg of paraffin wax embedded fixed tissues using the DNeasy Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA was eluted from the column with 50–100 µl of elution buffer and kept at –20°C until use. Amplification of the rearranged Ig heavy chain

Abbreviations: Ig, immunoglobulin; MALT, mucosa associated lymphoid tissue; PCR, polymerase chain reaction

Figure 1 Low magnification of a gastric low grade B cell mucosa associated lymphoid tissue lymphoma characterised by a dense and monomorphous lymphoid proliferation expanding in the gastric mucosa. Inset: a destructive pattern of the gastric lymphoma with a lymphoepithelial lesion.

Figure 2 Immunohistochemical staining with anticytokeratin antibody showing destructive lymphoepithelial lesions.

genes was performed by PCR in a 25 µl volume, using 2 µl of template DNA. Each reaction tube contained 10 pmols of FR3A¹ and JH² primers, 10mM Tris/HCl, 50mM KCl, 1.5mM MgCl₂, 250µM dNTPs, and 0.5 unit Taq DNA polymerase. The amplification protocol was as follows: 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 75 seconds. After 40 cycles, each PCR product was analysed by electrophoresis using a 2% agarose gel containing ethidium bromide, and the DNA was visualised under ultraviolet light. For each case, PCR amplification and agarose gel analysis was performed at least twice.

PATHOLOGICAL FINDINGS

The first gastric biopsy samples (1988) showed a mild chronic gastritis of the antrum and fundus in the presence of several *H pylori* organisms on Giemsa stain. One year later, histological examination of the second biopsy specimen revealed a dense and monomorphous lymphoid proliferation of centrocyte-like cells expanding in the gastric lamina propria of the fundus with destructive lymphoepithelial lesions of the glands (fig 1). However, only a few *H pylori* were still present. Biopsy specimens obtained 11 years later showed the persistence of the destructive lymphoid proliferation still limited to the lamina propria of the fundus, without signs of progression or blastic transformation. *Helicobacter pylori* were still present but were very rare.

Immunohistochemistry performed on the first series of antral and fundic mucosal biopsies (1988) showed that the lymphoid population consisted of a mixture of B and T cells, supporting the reactive nature of this population. In the last two series of biopsies (1989 and 2000), the lymphoid

Figure 3 PCR amplification of IgH gene rearrangement analysed by polyacrylamide gel electrophoresis. Lane C+, B cell lymphoma positive control. Lanes 1A, B, no monoclonal band in the biopsy from year 1988. Lanes 2A and 2B, single monoclonal reproducible band in the biopsy from year 1989. Lanes 3A and 3B, single monoclonal reproducible band in the biopsy from year 2000, different from that seen in 1989.

proliferation consisted of cells positive for the L26 antibody with a monoclonal IgG pattern and negative for the CD3 antibody. The recognition of lymphoepithelial lesions was facilitated by staining for cytokeratin (fig 2).

PCR of the lymphoid tissue showed a clonal rearrangement in the last two series of biopsies (1989 and 2000), whereas no clonal rearrangement was recognised in the first biopsy. Interestingly, the bands were of different sizes in the two series of biopsies (fig 3).

A low grade B cell MALT lymphoma was diagnosed based on morphological, immunohistochemical, and molecular genetic analysis.

DISCUSSION

We describe a case of low grade B cell MALT lymphoma of the stomach followed during 11 years, which showed no clinical or morphological progression, despite the fact that the patient was not treated for lymphoma or for *H pylori* infection. This case raises the problem of the role of *H pylori* in the progression of gastric MALT lymphoma. Furthermore, the indolence of the disease for such a long period of time brings into question the reactive or tumoral nature of the lymphoid proliferation.

Immunophenotyping by immunohistochemistry⁵ and the demonstration of immunoglobulin gene rearrangements, either by Southern blot analysis or by PCR, are commonly used as ancillary tests in the assessment of MALT lymphomas. However, clonal B cell populations have also been reported in reactive inflammatory tissues.^{6,7} This finding might be explained by the high sensibility of the PCR method, which is able to amplify very small clones. It was concluded that, although the presence of a clonal lymphoid population in an appropriate histological context would support a diagnosis of lymphoma, clonality cannot be considered as synonymous with malignancy.⁸ However, it has been shown in a recent study that Ig rearrangements observed in *H pylori* associated gastritis were not reproducible throughout the sequential sections carried out at different depths, whereas those associated with a low grade MALT lymphoma were.⁶ In our case, the histological destructive growth and the B immunophenotype of the lymphoid proliferation in the two last series of biopsies raised no doubt about the malignant nature of the lymphoid proliferation. Moreover, the Ig rearrangements were observed only in the biopsies where a lymphoma was suspected morphologically. In the first series of biopsies, where a reactive inflammatory process was observed, no rearrangement was

detected. Furthermore, the rearrangements were clearly defined and the same results were obtained in a second PCR control performed on each sample. Interestingly, the rearrangements were different in the two last series of biopsies after a follow up of 11 years, suggesting that a new lymphomatous clonal independent focus had emerged during the follow up of the MALT lymphoma. A coexisting clonal focus not seen in the first biopsy of MALT lymphoma and detected only in the second biopsy of MALT lymphoma might be another hypothesis. Although the clone discovered in the second biopsy was probably no longer present in the third biopsy, it is possible that the first clone remained, but only as a minor one. Indeed, because of the use of consensus primers for the PCR reaction, all the Ig heavy chain gene rearrangements cannot be amplified to the same degree. These hypotheses are supported by the findings of Ott *et al.*⁹ They described the presence, in two of 12 patients with MALT gastric lymphoma, of additional clonal B cell populations, and considered that they were independent foci.

The relation between *H pylori* associated gastritis and MALT lymphoma is well established and *H pylori* is now considered to be a risk factor for gastric MALT lymphoma.¹⁰ However, in a previously reported study of our own group,⁴ and in a study by another group,¹⁰ it was found that the frequency of *H pylori* infection is higher in MALT lymphomas restricted to the mucosa and submucosa than in more advanced cases. Moreover it was reported, as in our case, that the density of *H pylori* decreases significantly in patients with chronic gastritis who develop gastric MALT lymphoma preceded by B cell monoclonality, when compared with similar patients who do not develop MALT lymphoma.¹⁰ This suggests that *H pylori* infection tends to disappear with progression of the lymphoma and is probably associated more with the precursor or initial phase of gastric MALT lymphoma, and might not be necessary for sustained lymphoma cell proliferation.

Although the eradication of *H pylori* has resulted in clinical and histological remission, molecular genetic evidence of the neoplastic clone may persist in 40% of patients (14%),⁹ suggesting once more that *H pylori* eradication may not necessarily stop the disease progression. However, even the persistence of a clonal band does not indicate persistence of the lymphomatous disease because the morphology converts to normal and the clonal band disappears with time; in our case, there was neither endoscopic nor morphological MALT lymphoma regression.

In summary, this case report describes a patient with a lymphoid proliferation in the gastric mucosa that fulfilled the criteria for a B cell low grade MALT lymphoma, but who did not progress during 11 years, despite the absence of *H pylori* eradication. The detection of Ig rearrangements remains a useful tool for the diagnosis of gastric MALT lymphoma. PCR findings suggest that coexisting or new lymphomatous clonal independent foci may emerge during the follow up of MALT

Take home messages

- Despite the absence of *Helicobacter pylori* eradication, this patient with a B cell low grade mucosa associated lymphoid tissue (MALT) lymphoma of the stomach did not progress during 11 years
- This reaffirms the indolent nature of this lymphoma and supports the hypothesis that sustained *H pylori* infection by itself is not sufficient for tumour progression
- In the evolution of MALT lymphomas, different cell clones characterised by different immunoglobulin rearrangements may emerge

lymphomas. This case report illustrates the indolence of low grade MALT lymphoma of the stomach and supports the hypothesis that sustained *H pylori* infection is, by itself, not sufficient for tumour progression.

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