Detection of *Helicobacter pylori* associated antigen and heat shock protein 60 on follicular dendritic cells in the germinal centres of low grade B cell lymphoma of gastric mucosa associated lymphoid tissue (MALT)

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Abstract

Aims—To investigate the localisation of *Helicobacter pylori* antigens and the expression of human heat shock proteins (HSP) in stomachs affected by MALT lymphoma.

Methods—Surgically resected stomachs from 24 patients with MALT lymphoma were immunostained with anti-*H pylori* rabbit antibodies (ORP-1 and ORP-2) and anti-human HSP60 mouse monoclonal antibodies (mAb) (LK-1 and LK-2).

Results—Follicular dendritic cells of germinal centres in the stomachs affected by MALT lymphoma were immunostained with anti-*H pylori* polyclonal antibodies and with anti-human HSP60 mAb, as were the epithelial cells. None of the lymph node samples reacted.

Conclusions—Human HSP60, which cross reacts with anti-*H pylori* polyclonal antibodies, is often expressed on follicular dendritic cells in gastric MALT lymphoma tissues and may be aetiologically relevant to lymphomagenesis of MALT lymphoma.

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Low grade B cell lymphoma of gastric mucosa associated lymphoid tissue (MALT) is histologically characterised by a diffuse infiltrate of centrocyte-like cells, plasmacytic differentiation, and lymphoepithelial lesions. Germinal centres are invariably present in the lesions.¹ MALT lymphoma shows ongoing mutations of the variable region of immunoglobulin heavy chain (IgH) genes, indicating the derivation from postgerminal centre memory B cells.² Memory B cells and plasma cells are generated from antigen specific B cells within germinal centres through a somatic hypermutation of immunoglobulin genes in association with antigens on follicular dendritic cells.³ However, the normal stomach is devoid of MALT and acquires lymphoid follicles after *Helicobacter pylori* infection.⁴ There is evidence showing a close association between *H pylori* gastritis and the development of gastric MALT lymphoma⁵; the regression of MALT lymphomas after the eradication of *H pylori*^{6,7} and its relapse after

reinfection^{8,9} have been reported. The presence of clonal IgH gene rearrangements¹⁰ and the substantial number of lymphocytes and plasma cells with the immunoglobulin idiotype of MALT lymphoma¹¹ in chronic gastritis associated with *H pylori* also support the hypothesis that *H pylori* is involved in the pathogenesis of low grade gastric MALT lymphomas. In this report, we describe the pathogenic implications of heat shock protein (HSP) from *H pylori* and humans in the development of gastric low grade MALT lymphoma.

Methods

TISSUES

The stomachs were surgically resected from 24 patients with gastric low grade MALT

Figure 1 Western blotting analysis of the reactivity of anti-human HSP60 monoclonal antibodies (LK-1, lanes 1 and 2; LK-2, lanes 3 and 4) with human HSP60 (lanes 1 and 3) and the extract of *H pylori* (lanes 2 and 4). LK-2 cross reacts with a protein of about 66 kDa in *H pylori* extract, probably corresponding to HSP60 of *H pylori*.

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Figure 2 Immunohistochemical staining of gastric lymphoid tissue of MALT lymphoma with a rabbit anti-*H. pylori* antibody (ORP-1) and an anti-human HSP60 monoclonal antibody (LK-2). Top left: The reactive germinal centre and some epithelial cells are positively immunostained (ORP-1, $\times 72$). Top right: Immunoreaction products are localised on follicular dendritic cells in the germinal centre showing colonisation (ORP-1, $\times 290$). Bottom left: Double immunostaining for follicular dendritic cells (CD35) and *H. pylori* antigen (ORP-1). Follicular dendritic cells, red; *H. pylori* antigen, blue ($\times 290$). Bottom right: Immunostaining for human HSP60. Frozen section (LK-2, magnification $\times 290$).

lymphoma and from five patients with gastric ulcer infected with *H. pylori*. Formalin fixed, paraffin embedded specimens were obtained from all of the patients. Frozen sections were available from three cases of MALT lymphoma. Lymph node samples from patients with gastric MALT lymphoma (five cases), colon cancer (seven cases), prostatic cancer (two cases), lung cancer (three cases), necrotising lymphadenitis (two cases), abscess forming granulomatous lymphadenitis (three cases), and reactive lymphadenitis (two cases) were also used.

ANTIBODIES

Anti-*H. pylori* polyclonal antibodies were raised in rabbits by immunising them with sonicated whole cells of *H. pylori* intravenously and subcutaneously. Purified IgG antibodies (ORP-1 and ORP-2) were obtained from the pooled sera and used at a dilution of 1:100. Peroxidase conjugated and non-conjugated rabbit antibodies against human Ig γ chain, Ig α chain, and Ig μ chain were purchased from Dako (Copenhagen, Denmark).

Mouse monoclonal antibodies (mAb) against the human HSP60 (LK-1 and LK-2) were obtained from Stress Gen (Victoria, British Columbia, Canada) and used at a dilution of 1:100. Western blotting analysis showed that

LK-2 also cross reacted with HSP60 of *Escherichia coli* (Gro EL) and *H. pylori* (fig 1).

IMMUNOSTAINING

H. pylori antigens were immunostained by the ordinary avidin-biotin complex (ABC) method using ORP-1 or ORP-2 as a primary antibody and horseradish peroxidase conjugated anti-rabbit IgG goat F(ab')₂ (Zymed Lab Co, San Francisco, California, USA) as a secondary antibody. For the immunohistochemical detection of the human HSP60, acetone fixed frozen sections were also examined by the ABC method using LK-1 or LK-2 as a primary antibody and peroxidase conjugated rabbit anti-mouse Ig (Dako) as a secondary antibody. Follicular dendritic cells were detected by anti-CD35 mouse mAb and a catalysed signal amplification system (Dako). Alkaline phosphatase labelled secondary antibody was used for the double staining of HSP60.

Results

Paraffin embedded tissue sections from the stomachs affected by low grade MALT lymphoma were immunostained with the rabbit anti-*H. pylori* antibodies ORP-1 and ORP-2. These antibodies reacted with bacteria themselves in 22 of the 24 cases. Furthermore, the reaction products were localised in the

cytoplasm and the luminal surface of some foveolar and glandular epithelia, although the reactivity was variable among the cells (fig 2, top left). Interestingly, these antibodies also reacted with the non-neoplastic germinal centres in 14 of the 24 cases (fig 2, top left). Some of the follicles showing follicular colonisation, consisting of an excess of centrocyte-like cells, also contained positive reaction products (fig 2, top right). The germinal centres of the resected stomachs in one of the five patients with an *H pylori* infected gastric ulcer also reacted. Preimmune rabbit serum reacted with none of these samples. Immunohistochemical reactivity in the germinal centres showed the reticular networks which are thought to correspond to follicular dendritic cells. The immunoreactivity of follicular dendritic cells was also confirmed by the double immunostaining for CD35 and for *H pylori* antigens (fig 2, bottom left). None of the lymph node samples obtained from the patients with MALT lymphoma and other inflammatory or neoplastic diseases reacted with ORP-1 or ORP-2. Anti-human HSP60 mAb (LK-1 and LK-2) reacted with GCs in two of the three patients with gastric MALT lymphoma from whom frozen sections were obtained, which were also immunostained with anti-*H pylori* polyclonal antibodies (fig 2, bottom right). On the other hand, anti-human HSP60 mAb did not react with germinal centres in three cases of reactive lymphoid hyperplasia used as a control.

Discussion

In this study, we showed that the *H pylori* related antigens are expressed in the germinal centres of gastric MALT lymphoma tissues. The staining pattern of the germinal centres strongly suggested that the antigen recognised with anti-*H pylori* antibodies is present on follicular dendritic cells; this was confirmed by the double immunostaining with anti-CD35 mAb and anti-*H pylori* polyclonal antibodies. The positive selection of B cells on the basis of their high affinity for the antigen takes place in the germinal centres, where the high affinity B cell mutants are primed to become effective antigen presenting cells and receive help from T cells in the context of an antigen specific, MHC restricted cognate interaction.³ MALT lymphomas are of memory B cell origin and are closely associated with *H pylori* infection,⁵ which induces reactive follicles in the stomach. These results suggest that MALT lymphoma may be driven to proliferate by the *H pylori* antigens on the follicular dendritic cells. However, the follicular dendritic cells of the germinal centres were immunostained not only with rabbit anti-*H pylori* polyclonal antibodies, but also with the anti-human HSP60 mAb LK-1 and LK-2. A rabbit antiserum against *H pylori* HSP60 has been shown to cross react with the human HSP60,¹² and LK-1 recognises only the human HSP60. Therefore one of the antigens expressed on follicular dendritic cells may be the human HSP60 induced after *H pylori* infection. However, *H pylori* HSP60 and other *H pylori* related antigens are also trapped on

follicular dendritic cells. We found that polyclonal antibodies against *H pylori* and anti-HSP mAb also reacted with gastric epithelial cells in addition to follicular dendritic cells, which is consistent with the findings of a previous report.^{13 14}

Both anti-idiotypic antibodies produced against MALT lymphoma cells and immunoglobulin secreted from lymphoma cells reacted with normal tissue components.¹⁵ The germlines of VH genes used by MALT lymphoma are frequently found in autoreactive antibodies.² Autoantibodies are often detected in patients with *H pylori* infected atrophic gastritis.¹⁶ We recently found that sera from about 80% of the patients with MALT lymphoma reacted with recombinant human HSP60 (in preparation). Our study suggests that one of the autoantigens recognised by MALT lymphoma cell immunoglobulin may be HSP60, which may be aetiologically relevant to lymphomagenesis of MALT lymphoma.

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