Antibodies to human gastric epithelial cells and heat shock protein 60 in Helicobacter pylori positive mucosa associated lymphoid tissue lymphoma

Y Kawahara, K Yokota, M Mizuno, N Yunoki, T Uesu, H Okada, K Kobayashi, Y Hirai, K Oguma, T Tsuji

Abstract
Background—Development of gastric mucosa associated lymphoid tissue (MALT) lymphoma is thought to be closely associated with host immune reactions to Helicobacter pylori.
Aim—To investigate humoral immune responses in patients with MALT lymphoma to antigens shared by H pylori and human gastric epithelial cells.
Methods—Sera were obtained from H pylori positive patients with MALT lymphoma (n = 11) or other gastroduodenal diseases (peptic ulcer, n = 40; non-ulcer dyspepsia, n = 20) and from H pylori negative healthy control subjects (n = 10). Antibodies to HGC-27 human gastric epithelial cells and human recombinant heat shock protein (Hsp) 60 were examined using an enzyme linked immunosorbent assay (ELISA) and immunoblotting.
Results—Antibody titres to HGC-27 cells were significantly elevated in H pylori positive patients with MALT lymphoma when compared with titres in patients with other gastroduodenal diseases and in healthy subjects. Immunoblotting of sera from patients with MALT lymphoma often detected a band with a molecular mass corresponding to Hsp60, and both ELISA and immunoblotting showed elevated antibody titres to the recombinant human Hsp60. Antigenic similarity between Hsp60 and H pylori HspB was documented by immunoblotting experiments.
Conclusions—Autoantibodies reactive with host gastric epithelial cells are often increased in MALT lymphoma, and Hsp60 is a major target antigen. Immune responses induced by immunological cross reactivity between H pylori HspB and human Hsp60 in gastric epithelium may be involved in the development of MALT lymphoma.

Keywords: Helicobacter pylori; mucosa associated lymphoid tissue lymphoma; heat shock protein; autoantibody

Accumulating evidence suggests that Helicobacter pylori is involved in the pathogenesis of gastric lymphoma of mucosa associated lymphoid tissue (MALT) type. Antigenic mimicry between H pylori and the host mucosa may induce host autoimmune responses which lead to the development of the disease. Heat shock protein (Hsp) is one candidate for the cross reacting antigen(s). Hsps are a family of proteins induced by cell stress, such as microbial infections, and are conserved in both prokaryotic and eukaryotic cells. Hsps with identical antigenic structures have been shown by immunohistochemistry in H pylori bacterial cells and gastric epithelial cells of patients infected with H pylori. In this study, we investigated host humoral immune responses to H pylori which may lead to the production of autoreactive antibodies. We measured antibodies against gastric epithelial cells and human Hsp60 in patients with MALT lymphoma, peptic ulcers, and non-ulcer dyspepsia using an enzyme linked immunosorbent assay (ELISA) and immunoblotting.

Patients and methods

Patients
Sera were obtained from 71 H pylori positive patients, including 11 with MALT lymphoma (six women and five men; mean age 59.8 years), 20 with gastric ulcer (three women and 17 men; mean age 46.3 years), 20 with duodenal ulcer (10 women and 10 men; mean age 30.4 years), and 20 with non-ulcer dyspepsia (six women and 14 men; mean age 41.5 years). Sera were also obtained from 10 healthy volunteers (seven women and three men; mean age 37.9 years old). Informed consent was obtained from each patient and healthy volunteer.

Diagnosis was made based on findings of endoscopic examination and histological examination of gastric biopsy or gastrectomy specimens. Histology of MALT lymphoma was assessed according to the REAL classification. Tissue specimens were analysed immunohistochemically using antibodies to IgG, IgA, CD20, CD3, CD75, and bcl-2 (Dako Japan, Tokyo, Japan). Immunoglobulin heavy chain gene rearrangement was examined by Southern blotting and hybridisation with a probe to the joining region of the immunoglobulin heavy chain gene. All the lymphomas showed lymphoepithelial lesions histologically and an immunophenotype compatible with that of MALT lymphoma. Monoclonality of the tumours was shown either by light chain restriction immunohistochemically or by molecular

Abbreviations used in this paper: MALT, mucosa associated lymphoid tissue; Hsp, heat shock protein; ELISA, enzyme linked immunosorbent assay; PBS, phosphate buffered saline.
genetic examination. All the lymphomas were low grade.

Infection with *H pylori* was documented by culture, rapid urease test, and histology of gastric biopsy specimens and by the presence of serum antibodies to *H pylori* by ELISA. *H pylori* was detected in the gastric mucosa of all patients whose sera were positive for anti-*H pylori* antibodies. Negative *H pylori* infection of the control healthy volunteers was defined by seronegativity to *H pylori*.

ELISA FOR THE DETECTION OF ANTIBODIES TO HUMAN GASTRIC EPITHELIAL CELLS AND HUMAN HSP60

Serum antibodies to gastric epithelial cells were measured by cell ELISA. HGC-27 human gastric cancer cells were cultured in 96-well microtitre plates. The cells were washed once gently with phosphate buffered saline (PBS), fixed with 2% formalin in PBS for two hours at room temperature, and washed twice with PBS. After blocking with PBS containing 10% skimmed milk, plates were incubated with 1:100 diluted serum for two hours, and washed with PBS containing 0.05% Tween 20. Then peroxidase labelled rabbit anti-human IgG antibody (Dako Japan) was added, and the plates were incubated for two hours. After being washed, the well contents were allowed to react with o-phenylenediamine in citrate buffer, pH 5.5. Absorbance at 490 nm was measured by an ELISA plate reader.

For the detection of antibodies to human Hsp60, wells of microtitre plates were coated with a recombinant human Hsp60 protein (Stress Gen, Victoria, British Columbia, Canada) (10 mg protein/well) and blocked with PBS containing 10% skimmed milk. The plates were incubated with 1:100 diluted serum for two hours at room temperature and washed with PBS containing 0.05% Tween 20, and bound human antibodies were detected as described above.

IMMUNOBLOTTING

Sonicated *H pylori* extracts, HGC-27 cells, and the recombinant human Hsp60 were dissolved in 1% sodium dodecyl sulphate containing 5% 2-mercaptoethanol and separated by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (10% gel). The separated proteins...
Using ELISA with plates coated with recombinant human Hsp60. *p<0.001 (Student’s t test).

Dyspepsia, and control healthy subjects. Serum antibodies to human Hsp60 were measured in each group. Autoantibodies to HGC-27 cells were present in the serum of most patients with MALT lymphoma, and the antibody titres in these patients were significantly higher than those in the other patient groups and the healthy subjects (*p<0.001).

To define the target molecule(s) of the autoantibodies to HGC-27 cells, we performed immunoblotting using HGC-27 cells (fig 2). LK-2 anti-human Hsp60 mouse monoclonal antibody detected a band of molecular mass about 60 kDa, suggesting that HGC-27 cells constitutively express Hsp60. Serum from patients with MALT lymphoma detected various bands, and the band of molecular mass corresponding to Hsp60 was commonly observed in patients with MALT lymphoma (fig 2A). In contrast, serum from the other patient groups and the healthy control group rarely detected a band corresponding to Hsp60 (fig 2B).

Next, we tried to establish that the patients with MALT lymphoma had circulating antigens to Hsp60, using immunoblotting and ELISA with the human recombinant Hsp60. On immunoblotting, 10 of 11 sera (91%) from patients with MALT lymphoma recognised a band corresponding to Hsp60 (fig 3), whereas sera from the other patient groups and the healthy control detected it significantly less (0–20%, p<0.01) (table 1). On ELISA, sera from patients with MALT lymphoma showed significantly higher antibody titres to Hsp60 than sera from the patients without MALT lymphoma and from the healthy control group (p<0.001) (fig 4).

CROSS REACTIVITY OF LK2 ANTI-HUMAN HSP60 ANTIBODY TO H PYLORI

LK-2 monoclonal antibody to the human Hsp60 recognised bands of 60 kDa in both H pylori and HGC-27 cells, which probably correspond to H pylori HspB12–14 and human Hsp60 respectively (fig 5).

Discussion

In this study, we found that serum antibody titres to HGC-27 human gastric epithelial cell were significantly elevated in H pylori positive patients with MALT lymphoma when compared with titres in H pylori positive patients who had other gastroduodenal diseases and in healthy subjects without H pylori infection. Our
immunoblot experiments showed that sera from patients with MALT lymphoma often detected a band with a molecular mass corresponding to that of Hsp60. Moreover, elevated antibody titres to the recombinant human Hsp60 were shown by ELISA and immunoblotting. Thus Hsp60 is probably a major antigen recognised by the anti-HGC-27 antibodies present in serum of patients with MALT lymphoma.

The LK-2 anti-human Hsp60 antibody recognises the epitope encoded by amino acid residues 383 to 419 of the human Hsp60 protein. We found that this antibody also detected a band corresponding to bacterial HspB in H pylori extracts, suggesting that a common epitope(s) is present in human Hsp60 and its bacterial homologue, HspB. Thus infection of H pylori may induce antibodies against bacterial HspB which cross react with a host Hsp60 through the molecular mimicry of these proteins, leading to the elevation of antibodies to HGC-27 cells in patients with MALT lymphoma. We have recently observed staining with LK-2 anti-Hsp60 antibody in follicular dendritic cells in germinal centres of gastric mucosa from patients with MALT lymphoma, suggesting that the antigen recognised by the anti-Hsp60 antibody which originates from either the bacteria or the host cell is presented to immune cells in this disease.

We found that the immune response to Hsp60 was closely associated with MALT lymphoma. Although the association of MALT lymphoma with H pylori strains expressing the CagA protein has been reported, no specific differences in HspB protein amino acid sequences between bacterial strains from MALT lymphoma and those from other gastroduodenal diseases have been demonstrated. However, production of antibody to Hsp60 was less marked in other diseases with H pylori infection. Undefined bacterial changes or host genetic backgrounds of immune responses to the bacterial infection may lead to enhanced immune responses to Hsp60 in patients with MALT lymphoma, but the reason for this specific response awaits clarification.

Induction of immune responses to host components may lead to tissue injury of an autoimmune nature. It is well known that gastric mucosal lesions of MALT lymphoma are often resistant to ordinary peptic ulcer treatment—that is, control of acid secretion. Immunological responses to H pylori of both neoplastic B cells and non-neoplastic T cells have been reported in MALT lymphoma. In addition, gastric ulcers developed in severe combined immunodeficient mice transplanted with peripheral blood mononuclear cells from patients with MALT lymphoma when H pylori was orally inoculated. These observations suggest that the development of gastric mucosal damage in patients with MALT lymphoma involves host immune responses to H pylori. Hsp60 may be one of the target molecules.

In this study, a few H pylori infected patients with gastric disease other than MALT lymphoma also had elevated IgG titres to anti-HGC-27 cells and Hsp60. We are currently following these patients to see whether they will develop gastric MALT lymphoma. In addition, the common association of anti-Hsp60 and anti-HGC-27 antibodies with MALT lymphoma suggests that measurement of these antibodies may provide a new diagnostic indicator for the disease.

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