Immune response to heat shock protein of *Helicobacter pylori* – a candidate as a vaccine component

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Abstract. The reactive epitope of heat shock protein 60 (HSP60) of *Helicobacter pylori* to its monoclonal antibody (H9) was determined, and its synthesized peptide designated pH9 was used for ELISA. The patients with *H. pylori* infection had significantly lower titers of pH9 antibody than did uninfected patients. In C57BL/6 mice immunized intraperitoneally with the pH9 peptide with Freund's complete adjuvant (FCA), the number of *H. pylori* organisms colonizing the stomach was significantly lower than that in mice immunized with FCA only. These results suggest that HSP60 of *H. pylori* is effective in protection against *H. pylori* infection and might be a good candidate as a vaccine component.

Introduction

Heat shock proteins (HSP), highly conserved proteins found in all prokaryotic and eukaryotic cells, are induced by a variety of environmental stresses, such as temperature change, inflammation, viral infection and malignant transformation.1,2 The HSP60 family of chaperonins is thought to be immunodominant antigens and to facilitate folding, unfolding, and translocation of polypeptides as well as the assembly and disassembly of oligomeric protein complexes.3,4 To understand the possible role of the immune response to a cross-reactive epitope on *Helicobacter pylori* HSP60, we determined the epitope recognized by the H9 monoclonal antibody (MAb) to HSP60 and analyzed the human humoral immune response against its epitope region. The protective effect of immunizing mice with the epitope region on *H. pylori* infection was also investigated.

Materials and Methods

**Human sera**

349 *H. pylori* positive sera and 200 *H. pylori* negative sera were used.

**Construction of E. coli expressing H. pylori HSP60**

DNA fragments of different length encoding *H. pylori* HSP60 were amplified as described before.5 The PCR products were integrated into a plasmid, pEX, capable of producing a fused protein with β-galactosidase.

**Preparation of MAb**

MAb H9, which reacts with *H. pylori* HSP60, was previously established.6 The 60-kDa antigen derived from *H. pylori* TK1029 strain was partially purified by extraction from separating gels after SDS-PAGE.

**Peptide ELISA**

AquaBind 96-well microplates were coated with pH9, oligopeptide reactable with H9 MAb at a concentration of 0.3 to 10 µg/well. ELISA was performed as described before.5

**Animal experiment**

Specific-pathogen free C57BL/6 mice (5-week-old females) were intraperitoneally immunized five times on a weekly schedule with pH9 peptide plus Freund's complete antigen (FCA). One week after the last immunization, the mice were orally infected 3 times daily with $5 \times 10^8$ cells of the *H. pylori* TK1402 strain. Two weeks after infection, the mice were sacrificed. Assessment of *H. pylori* colonization in mouse stomach was performed by microaerophilic incubation as described before.6

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Table 1 Serum IgG Responses to HSP60 Homologues, Synthesized Peptide pH9, and H. pylori Whole Antigen as Measured by ELISA

<table>
<thead>
<tr>
<th>Antigen**</th>
<th>Optical density*</th>
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<tr>
<td></td>
<td>H. pylori infected (n = 349)</td>
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<tr>
<td>H. pylori HSP60</td>
<td>0.365 ± 0.013</td>
</tr>
<tr>
<td>pH9</td>
<td>0.278 ± 0.012</td>
</tr>
<tr>
<td>E. coli GroEL</td>
<td>0.405 ± 0.019</td>
</tr>
<tr>
<td>Human HSP60</td>
<td>0.039 ± 0.003</td>
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* Values are means ± standard errors of the means. n, number of patients. ** Each antigen was used at 100 ng/well. * Determined by Welch's unpaired t test. ** NS, not significant.

**Results**

Determination of an epitope recognized by H9 MAb on H. pylori HSP60

By immunoblot analysis using MAb H9 of various fusion proteins of different length of HSP60 and β-galactosidase, the epitope recognized by H9 MAb on H. pylori HSP60 was determined. The sequence of amino acids 189 to 203 (VEGMQFDRGYLSPYF) on H. pylori HSP60 was considered a candidate for the epitope recognized by H9 MAb. This amino acid sequence was synthesized and designated as pH9. In parallel, the amino acid sequence 463 to 477 (VNEVEKHEGFGFNA) non-reactive with MAb H9 was synthesized and designated as pCont.

Human humoral immune response against pH9

The serum samples from H. pylori-infected patients had significantly higher levels of serum IgG antibodies recognizing affinity-purified H. pylori HSP60 than did the sera of uninfected persons (Table 1). However, serum samples from uninfected subjects had lower titers of pH9 antibody than did those of uninfected subjects (p < 0.001) (Table 1).

Protective effect of immunizing mice with the pH9 peptide on H. pylori infection

The number of H. pylori colonizing the stomach mucosa of mice immunized with pH9 plus FCA was significantly lower than that in mice immunized with either pCont plus FCA or FCA only (Table 2).

**Discussion**

It has been reported that certain bacterial HSP60 (GroEL) proteins are able to induce a protective immune response against infection by Legionella spp. Noll et al. also reported the protective effect role of a cross-reactive epitope on HSP60 of Yersinia enterocolitica in murine yersiniosis. The present study showed that the humoral immune response against the pH9 peptide is dominant in uninfected patients, indicating that the humoral immune response to a cross-reactive epitope recognized by H9 MAb might be different from that to other epitopes on H. pylori HSP60. We also demonstrated that the immune response against a cross-reactive region on H. pylori HSP60 is associated with protection against H. pylori infection. These results suggest that pH9 might be a useful tool as a vaccine component for prevention of H. pylori infection.

**References**