Development of *Helicobacter pylori*-induced Gastric Carcinoma in Mongolian Gerbils

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Abstract

*Helicobacter pylori* is classified by IARC/WHO as a definite human gastric carcinogen, despite "inadequate experimental evidence." To obtain direct evidence concerning this relationship, we investigated the histopathological findings of gastric mucosa using a model of *H. pylori* infection in Mongolian gerbils. The animals were challenged p.o. with *H. pylori* ATCC-43504 and sacrificed at 6, 12, and 18 months after inoculation for histological examination. All inoculated animals were infected with *H. pylori*. Severe infiltration of the lamina propria by polymorphonuclear and mononuclear cells appeared in the lesser curvature of the antrum, with an increase in epithelial cell proliferation, and the infiltration extended to the body. Atrophic gastritis and focal intestinal metaplasia also appeared in the lesser curvature of the antrum mucosa at 6 months after inoculation. Intestinal metaplasia became severe, with dysplasia, after that. At 18 months after *H. pylori* inoculation, two of five infected animals showed three well-differentiated gastric cancers. The uninfected control animals showed no abnormal findings throughout the entire observation period. Here, it was confirmed that *H. pylori* infection alone causes gastric cancer in an animal model.

Introduction

A relationship between *Helicobacter pylori* infection and gastric cancer of both intestinal and diffuse types has been suggested in serological studies (1, 2), and two pathways have been proposed to explain the role of *H. pylori* infection and gastric cancer in both types (3). Evidence from an animal model is needed to prove this relationship. It has been reported that *H. pylori* infection causes atrophic gastritis and intestinal metaplasia in Mongolian gerbils (4, 5). Atrophy and intestinal metaplasia are considered precursors of intestinal-type gastric cancer (6). We observed histological changes in the stomachs of Mongolian gerbils infected with *H. pylori* and compared the results with those from uninfected control animals to investigate the relationship between *H. pylori* infection and gastric cancer.

Materials and Methods

Animals and Infection Model. Five-week-old male Mongolian gerbils weighing 30–40 g (Seac Yoshimoto Co. Ltd., Fukuoka, Japan) were fasted for 24 h and then fed chow (Oriental Yeast Co., Tokyo, Japan) and water *ad libitum* beginning 12 h after *H. pylori* inoculation. Mongolian gerbils were randomly divided into two groups and challenged p.o. with 10⁹ colony-forming units of *H. pylori* ATCC-43504, expressing the cagA gene and vacuolating cytotoxin, as described previously (5). Four or five animals in each experimental group were weighed and sacrificed under anesthesia with ether at 6, 12, and 18 months after inoculation. Immediately after killing, the blood was drawn from the heart, and serum was obtained and stored at -20°C until testing for anti-*H. pylori* antibody using ELISA method, as described previously (5). The stomach was quickly removed and used for histological examinations.

Experiments were performed according to the guidelines of Ethical Committee for Animal Experiments at Oita Medical University (Oita, Japan).

Histological Examinations. The stomach was fixed in 10% neutral and isotonic buffered formalin for 4 h. The stomach was cut up into eight longitudinal parts and embedded in paraffin. The 5-μm sections were stained with H&E, Giemsa, PAS, and Alcian blue (pH 2.5) stains and examined for inflammatory response with or without intestinal metaplasia and the presence of organisms.

Diagnosis of Dysplasia and Adenocarcinoma. Gastric dysplasia was diagnosed according to the following pathological criteria (7): increasing proliferation of the cells, abnormal morphology, and pleomorphism of the cells, architectural derangement of glands, and stromal changes. Gastric adenocarcinoma was diagnosed by the presence of atypical glands that invaded into the proper muscular layer.

Statistical Analysis. All values are expressed as the means ± SD. Data were analyzed by the Student's *t* test. Significance was defined as *P* < 0.05.

Results

Normal Histology of the Glandular Stomach of Mongolian Gerbils. Normal gastric mucosa of a Mongolian gerbil is shown in Fig. 1. All superficial epithelial cells, pyloric glands, and some cells of the fundic glands were stained by PAS stain. Pyloric glands were also stained by Alcian blue (pH 2.5). Superficial epithelial cells and fundic glands were not stained by Alcian blue stain. The uninfected control animals showed no abnormal findings throughout the entire observation period.

Body Weight and IgG Anti-*H. pylori* Antibody Titer. All inoculated animals showed high titers of the serum anti-*H. pylori* antibody, as compared to uninfected control animals, throughout the entire observation period, and the body weights of these animals were significantly lower than those of control animals at 6 months after inoculation (Table 1).

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**Table 1** Effect of *H. pylori* infection on body weight and anti-*H. pylori* antibody titer in Mongolian gerbils

<table>
<thead>
<tr>
<th>Group</th>
<th>Investigation time (months)</th>
<th>Body weight (g)</th>
<th>Antibody titer (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>86 ± 5.4</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>86 ± 11.4</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>100 ± 7.0</td>
<td>3.9 ± 0.4a</td>
</tr>
<tr>
<td><em>H. pylori</em></td>
<td>6</td>
<td>69 ± 9.6c</td>
<td>188 ± 89c</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>76 ± 13.4</td>
<td>2289 ± 2242d</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>82 ± 19.2</td>
<td>2707 ± 889e</td>
</tr>
</tbody>
</table>

*The abbreviations used are: PAS, periodic acid-Schiff; PMN, polymorphonuclear; MN, mononuclear.*

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Inflammatory Cell Infiltration and Gastric Ulcers. Microscopic examination showed severe infiltration of the lamina propria by PMN and MN cells in the antral mucosa. Although PMN and MN cell infiltration was first localized in the lesser curvature of antrum, it extended to the lesser curvature of body at 12 months after inoculation and to the greater curvature at 18 months. Gastric ulcers were continuously observed from 6 to 18 months after *H. pylori* inoculation (Table 2). They localized in area of the pyloric glands, and almost all of them penetrated the submucosa or the proper muscular layer. In addition, some penetrated the pancreas or liver.
Table 2. Histopathological changes of the gastric mucosa in stomachs of Mongolian gerbils infected with *H. pylori* ATCC-43504

<table>
<thead>
<tr>
<th>Histopathological findings</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis</td>
<td>5/5</td>
<td>4/4</td>
<td>5/5</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>4/5</td>
<td>3/4</td>
<td>5/5</td>
</tr>
<tr>
<td>Atrophic gastritis</td>
<td>4/5</td>
<td>4/4</td>
<td>5/5</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>2/5</td>
<td>3/4</td>
<td>5/5</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>0/5</td>
<td>2/4</td>
<td>5/5</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>0/5</td>
<td>0/4</td>
<td>2/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The uninfected control animals (*n* = 5 in each case) showed no abnormal findings.

<sup>b</sup> Three well-differentiated adenocarcinomas were observed in two of five animals.

**Atrophy and Intestinal Metaplasia.** The mucosa infiltrated with PMN and MN cells was hyperplastic first (Fig. 2), and the number of proper gastric glands was reduced after that. Atrophic gastritis and focal intestinal metaplasia appeared in the lesser curvature of antrum at 6 months after inoculation, and it extended to the body and greater curvature following inflammation. Intestinal metaplasia became severe and was associated with dysplasia at 12 and 18 months after *H. pylori* inoculation (Fig. 3).

**Occurrence of Gastric Cancer.** At 18 months after *H. pylori* inoculation, two of five infected animals showed three well-differentiated gastric cancers (Fig. 4). Two of the cancers did not develop directly from intestinal metaplasia but were associated with intestinal metaplasia at another location (Fig. 4, A–C). The other cancer developed from part of an intestinal metaplasia (Fig. 4D). Poorly differentiated gastric cancer was not observed.

**Discussion**

This study demonstrates gastric carcinogenesis of *H. pylori* in a Mongolian gerbil model. *H. pylori* infection first caused gastritis with hyperplastic glands of antrum. Some of these glands easily extended...
Helicobacter pylori-INDUCED GASTRIC CANCER

Fig. 4. A and B, microscopic views of the gastric mucosa of Mongolian gerbils at 18 months after H. pylori inoculation. Well-differentiated adenocarcinoma extended to the muscular layer. Atypical glands and nuclei and abnormal mitosis are seen. A. H&E stain; original magnification, ×10. B. H&E stain; original magnification, ×50. C, microscopic views of well-differentiated adenocarcinoma of Mongolian gerbils at 18 months after H. pylori inoculation. The proper muscular layer has been invaded by various sizes of glands with back-to-back appearance. H&E stain; original magnification, ×50. D, microscopic views of well-differentiated adenocarcinoma of Mongolian gerbils at 18 months after H. pylori inoculation. Atypical glands with atypical nuclei are seen in a metaplastic gland. This lesion seems to be an early stage of gastric cancer because it has not yet invaded the proper muscular layer. H&E stain; original magnification, ×50.

Atrophic gastritis and intestinal metaplasia appeared in the lesser curvature of the antral mucosa and extended to the body and greater curvature following inflammation. This agrees with reports that chronic atrophic gastritis and intestinal metaplasia are related to H. pylori infection (8) and that atrophy or intestinal metaplasia occurs at the lesser curvature and extends to greater curvature in the human (9, 10). Although some animals infected with H. pylori showed atrophic gastritis (11–13), intestinal metaplasia did not appear. A long-term follow-up study on atrophic gastritis in H. pylori-infected Japanese monkeys showed neither intestinal metaplasia nor gastric cancer (13). A model of Mongolian gerbils infected with H. pylori has been the only animal model for intestinal metaplasia, and it is similar to human gastric lesions caused by H. pylori infection. Intestinal metaplasia increased gradually, and dysplasia and well-differentiated gastric cancer occurred in this model. These consecutive changes support a pathway from H. pylori infection to intestinal-type gastric cancer in humans (6).

Here, two pathways for the occurrence of gastric cancer were seen: first, direct development from intestinal metaplasia, and second, indirect development from intestinal metaplasia, with intestinal metaplasia existing near the cancer. It is suggested that the two pathways exist between atrophic gastritis and well-differentiated gastric cancer.

Diffuse-type gastric cancer did not appear. However, these animals were infected with the ATCC-43504 strain at 5 weeks old and kept in the same environment. In humans, H. pylori strain, immune response of the host, and environment may differ. Further examination using various H. pylori strains and further studies of infection of H. pylori infection at various ages are needed.

On the other hand, gastric ulcers were continuously observed throughout the entire observation period and were localized in area of pyloric gland, as reported previously (4, 5). This suggests that gastric ulcer continues until eradication of H. pylori as well as H. pylori-induced gastric ulcers.

This study shows gastric carcinogenesis in a H. pylori-infected Mongolian gerbil model and confirms the IARC/WHO report that H. pylori is “a definite human gastric carcinogen” (14). In addition, this model is useful for the study of the mechanism of gastric carcinogenesis in H. pylori infection.

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References

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