Helicobacter pylori infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens

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Helicobacter pylori (Hp) is thought to be a stomach carcinogen from epidemiological findings. To determine the effects of infection with the bacteria on experimental carcinogenesis, a study of the glandular stomach of Mongolian gerbils (MGs) was performed. Male MGs were treated with N-methyl-N'-nitro-N-nitrosoguanidine followed by inoculation with Hp or infected with Hp followed by N'-nitro-N-nitrosoguanidine administration. Animals were killed at week 50, and their excised stomachs underwent microbiological and histopathological examinations. In addition, a serological investigation was performed. The incidences of adenocarcinomas were significantly higher in animals treated with 60 or 300 p.p.m. N-methyl-N'-nitro-N-nitrosoguanidine for 10 weeks followed by Hp inoculation or Hp followed by 20 p.p.m. N-methyl-N'-nitro-N-nitrosoguanidine for 30 weeks than in the respective controls. Moreover, tumour-bearing animals had higher titres of anti-Hp antibodies than tumour-free animals. Of interest was the finding that a dose of 100 p.p.m. N-methyl-N'-nitro-N-nitrosoguanidine given to infected gerbils eradicated the Hp in about half the animals, with a concomitant reduction in the promoting effect. No tumours were found in animals treated with 60 or 300 p.p.m. N-methyl-N'-nitro-N-nitrosoguanidine or non-treated gerbils. Hp infection enhances glandular stomach carcinogenesis in MGs treated with N-methyl-N'-nitro-N-nitrosoguanidine. Animals with high titres of anti-Hp antibodies are at greatest risk of developing neoplasms.

Introduction

A large amount of epidemiological evidence has accumulated indicating a significant relationship between Helicobacter pylori (Hp) infection and chronic gastritis (1,2), peptic ulcers (3), intestinal metaplasia (4,5), and adenocarcinoma (6–8) or lymphoma (9) development. In 1994, the World Health Organization/International Agency for Research on Cancer concluded that ‘Hp is a definite carcinogen’ based on the epidemiological findings (10). However, some studies have cast doubt on the link between Hp infection and an elevated risk of stomach cancer (11,12) and whereas eradication measures have attracted attention (13–16), the pathogenic role of Hp remains unclear. Hp infection almost always results in chronic antral gastritis, but only a proportion of patients develop stomach cancers. Some Hp strains may be more ‘carcinogenic’ than others, but host factors may also be important (17–19). For detailed analysis of the role of Hp in stomach carcinogenesis, it is essential to establish a small animal model. In Mongolian gerbils (MGs), Hp infection, chronic active gastritis, peptic ulcers and intestinal metaplasia closely mimic those in man (20). We have established an experimental model of stomach carcinogenesis in MGs using a chemical carcinogen (21) and demonstrated that Hp infection exerts an enhancing effect on tumour development in animals treated with N-methyl-N-nitrosourea (MNU) (22). The present study was performed to explore the influence of Hp infection on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced stomach carcinogenesis in MGs. In addition, variation in host serological response between tumour-bearing and tumour-free animals was also investigated.

Materials and methods

Chemicals

MNNG (Tokyo Kasei Kogyo, Tokyo, Japan) was dissolved in distilled water at concentrations of 300, 100, 60 and 20 p.p.m., and freshly prepared three times per week. The solutions were given as drinking water in light-shielded bottles ad libitum.

Bacteria

Hp (ATCC 43504; American Type Culture Collection, Rockville, MD) were inoculated on Brucella agar plates (Becton Dickinson, Cockeysville, MD) containing 7% (v/v) heat-inactivated fetal bovine serum and incubated at 37°C under micro-aerobic conditions using Anaero Pack Campylo (Mitsubishi Gas Chemical, Tokyo, Japan) at high humidity. Two days later, the bacteria grown on the plates were collected with inoculating loops, dissolved in 0.5 ml of Brucella broth (Becton Dickinson), and introduced into Brucella broth supplemented with 7% (v/v) heat-inactivated fetal bovine serum in tissue culture flasks with Vent caps and incubated under the same conditions for 24 h. The broth cultures of Hp were checked by phase contrast microscope for bacterial shape and mobility. Samples containing ~1.0×108 colony-forming units ml/(0.8 ml) were used as the inoculum, delivered intragastrically using an oral catheter after fasting for 24 h. Four hours thereafter, the animals were given access to water and feed again.

Animals

A total of 245 specific pathogen-free male, 7-week-old MGs (Meriones unguiculata, MGS/Se; Seac Yoshitomi, Fukuoka, Japan), were housed in steel cages on hard wood chip bedding in an air-conditioned biohazard room for infection, with a 12 h light–12 h dark cycle. They were given food (Oriental MF; Oriental Yeast, Tokyo, Japan) irradiated with 30 kGy γ-rays and autoclaved distilled water ad libitum. The experimental design described below was approved by the Animal Care Committee of the Aichi Cancer Center Research Institute.

Abbreviations: Hp, Helicobacter pylori; MG, Mongolian gerbil; MNU, N-methyl-N-nitrosourea; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; PBS, phosphate-buffered saline.

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Protocol of the study.

Fig. 1. Protocol of the study. 

Experiment I. One-hundred-and-three gerbils were divided into four groups. 

- Group 1: MNNG (300 mg/kg) + Hp. 
- Group 2: MNNG (300 mg/kg) + Brucella broth. 
- Group 3: MNNG (60 mg/kg) + Hp. 
- Group 4: MNNG (60 mg/kg) + Brucella broth. 

After completion of MNNG administration they were given autoclaved distilled water and, 1 week later, Hp was inoculated (groups 1 and 3). Groups 2 and 4 received Brucella broth without Hp. 

Experiment II. A total of 102 gerbils was divided into four groups. One week after inoculation with Hp (groups 5 and 7) or Brucella broth without Hp (groups 6 and 8), they were given MNNG in their drinking water at concentrations of 100 p.p.m. (groups 5 and 6) or 20 p.p.m. (groups 7 and 8) for 30 weeks continuously. After completion of MNNG administration, they were given autoclaved distilled water. The setting of doses of MNNG was made taking account of the period of MNNG administration and eradication activity of MNNG for Hp. In order to provide an equal total intake of MNNG, doses of MNNG in experiment II were one-third of that of experiment I. Lower doses of MNNG following Hp infection might have no eradication activity for Hp. 

Experiment III. Hp was given by intragastric intubation to 20 gerbils (group 9) after they had been subjected to 24 h fasting. Four hours after inoculation, they were allowed free access to water and feed again. As non-carcinogen controls for experiments I, II and III, 20 gerbils (group 10) were given Brucella broth without Hp (Fig. 1). 

Experiments I and II were run simultaneously in the same room, and experiment III was run at a different time, but in the same room. The experimental animals were weighed every week. Necropsies were performed on all animals which died or were killed upon becoming moribund. At experimental week 30, five animals in group 1, 3, 5 and 7 were killed, and checked for the status of Hp infection. 

After 24 h fasting, all animals were subjected to deep ether anaesthesia, laparotomized and exsanguinated from the inferior vena cava, with excision of their stomachs at the 50th experimental week. 

Histopathological examinations 

The excised stomachs were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.2), or 95% ethanol containing 1% acetic acid and cut into ~16 strips, processed by standard methods and embedded in paraffin. Tissues were sectioned at 5 μm for staining with haematoxylin and eosin (H&E), alcian blue-periodic acid-Schiff (AB-PAS) and by immunohistochemistry for Hp (anti-Hp serum; Dako, Copenhagen, Denmark). 

Histopathological findings were classified as follows: (1) active chronic gastritis characterized by severe infiltration of inflammatory cells; (2) ulcer; (3) hyperplasia; (4) submucosal proliferation characterized by invagination of glands into submucosal layer with a little or no cell atypia; (5) intestinal metaplasia; and (6) adenocarcinomas. Adenocarcinomas of the glandular stomach were classified into well differentiated lesions characterized by tubular structures with cellular atypia, poorly differentiated tumours characterized by little tendency to form glandular structures with severe cellular atypia and signet ring cell carcinomas characterized by isolated tumour cells containing abundant amounts of mucin. To avoid counting reactive changes as neoplasms, well differentiated adenocarcinomas were diagnosed on the criterion of presence of infiltration at least to the muscle layer. 

Inflammation of the glandular stomach was classified into grade 1, characterized by slight infiltration of inflammatory cells, and grade 2, characterized by inflammation observed in the gastric mucosa. 

Hp detection by culture 

For detection of Hp infection, ~30 mm² samples of stomach mucosa from the greater curvature, containing both fundic and pyloric glands, were homogenized with 1 ml of Brucella broth and used for culture of Hp. Aliquots of 0.1 ml were then inoculated on segregating agar plates for Hp (Eiken Chemical, Tokyo, Japan) and incubated at 37°C under micro-aerobic conditions at high humidity for 6 days. 

Anti-Hp antibody 

Blood samples containing a small amount of ethylendiaminetetra-acetic acid (EDTA) were centrifuged at 8000 r.p.m. for 5 min to isolate sera, which were then stored at ~80°C. Using MG sera thus obtained, anti-Hp IgG antibody (GAP-IgG; Biomerica, Newport Beach, CA) was measured by the enzyme-linked immunosorbent assay (ELISA). An ELISA system was developed in the medical laboratory of Shinshu University to quantitate serum anti-Hp IgG of MG using anti-MG IgG antibody. The titres of antibody were expressed using an arbitrary index (AI). An AI > 10.0 indicated the presence of Hp antibodies. To define this index, a reference serum was prepared by pooling the sera of several anti-Hp IgG-positive gerbils. The reference serum was diluted serially from 1:100 to 1:3200 with PBS (pH 7.4) containing 4% bovine serum albumin, and the amount of anti-Hp IgG corresponding to 1:3200 was expressed as the reference value of 1.0 AI. Microwell strips coated with Hp antigens of GAP-IgG kit (Biomerica) were used. Aliquots of 100 μl of reference serum or 1:200 diluted serum were added to the wells, and the plates incubated for 1 h at room temperature. After washing, 100 μl of horseradish peroxidase conjugated anti-gerbil IgG (diluted 1:1500 in PBS containing 0.05% Tween 20) was then added and the plates incubated for 30 min at room temperature. The plates were washed and incubated with 100 μl of substrate (0.35 mg/ml 3,3',5,5'-tetramethylbenzidine, 0.15 mg/ml H₂O₂) for 10 min. After stopping the reaction with 1 N HCl, the colour was read at 450 nm. The anti-Hp IgG value of each serum was determined from a standard curve of calibrator. 

Statistics 

The two-tailed t-test or Mann–Whitney’s U-test were applied to establish the significance of differences in body weight distributions and titres of anti-Hp antibodies. Survival curves were calculated by the Kaplan–Meier method and differences were evaluated using the log-rank test. The adenocarcinoma incidences were assessed by Fisher’s exact probability method. 

P-values <0.05 were considered to be statistically significant. 

Results 

Survival and total intake of MNNG 

Survival curves for MGs in groups 1–8 are shown in Figure 2. Survival rates for each group were >80% except for group 6, with no differences between groups 1, 3, 5 and 7, and their respective control groups 2, 4, 6 and 8. All MGs in groups 9 and 10 survived until week 50. The body weights in groups 1–10 at experimental week 50 were 81.30 ± 2.69, 85.78 ± 2.48, 84.49 ± 2.83, 93.83 ± 2.24, 91.05 ± 2.24, 81.29 ± 2.55, 102.43 ± 2.42, 97.25 ± 2.12, 92.20 ± 3.87 and 112.5 ± 1.09 g (average ± SE), respectively. The body weights of MGs in group 3 were significantly lower than those in group 4, and those in group 5 were higher than those in group 6. 

Total intakes of MNNG per MG are given in Tables I and II. 

Hp infection and inflammation 

In experiment I at week 50, the glandular stomachs were oedematous with haemorrhagic spots and erosions observed macroscopically in two-thirds of MGs in groups 1 and 3. Inflammation was histological grade 1 in 10 MGs and grade 2 in the other animals in group 1. Among 25 MGs in group 3, 5 animals had glandular stomach inflammation classified as grade 1. The inflammation in all animals of groups 2 and 4 was grade 1. 

In experiment II at week 50, the glandular stomachs demonstrated macroscopically evident inflammation in half of MGs in group 5 and all but one in group 7. Histological grade of inflammation was grade 1 in 11 MGs and grade 2 in the other animals in group 5. Among 25 MGs in group 7, only one
animal had glandular stomach inflammation, classified as grade 1. The inflammation of glandular stomachs of all animals in groups 6 and 8 was grade 1. Moreover, all animals negative for Hp immunohistochemistry had little or no inflammation, classified as grade 1 when present.

In experiment III at week 50, the glandular stomachs of MGs in group 9 were macroscopically oedematous, with haemorrhagic spots and erosions. Histologically, in 18 of 20 MGs, glandular stomach epithelium showed hyperplastic changes (Figure 3a) with variable degrees of multifocal cystic glandular dilatation and erosion. The bases of the glands penetrated the muscularis mucosae multifocally (classified by submucosal proliferation, Figure 3b). There was marked infiltration, predominantly of lymphocytes and some macrophages, as well as neutrophils in the lamina propria and submucosa, with frequent formation of lymphoid follicles. Intestinal metaplasia was also noted in 17 among 20 gerbils in group 9 (Figure 4a). Immunohistochemistry demonstrated the existence of Hp in inoculated MGs (Figure 4b). In all animals of group 10, the inflammation was grade 1 and no active chronic gastritis, ulcers, hyperplasia, submucosal proliferation or intestinal metaplasia were observed.

Incidence of induced glandular stomach adenocarcinomas

The incidences of adenocarcinomas of the glandular stomach are summarized in Tables I and II. Macroscopically, most tumours of animals in groups 1 and 3 demonstrated craters like type 2 stomach cancers in human, but about half of the tumours in group 7 were without craters like type 1 stomach cancers. Tumours were mostly found in the pyloric mucosa adjacent to the fundic region, and comprised well differentiated (Figure 5) and poorly differentiated adenocarcinomas (Figure 6), as well as signet ring cell carcinomas (Figure 7). The incidences of adenocarcinomas in groups 1, 3 and 7 were significantly higher than those in groups 2, 4 and 8, respectively. Only two of the 12 adenocarcinomas in group 1 and two of four adenocarcinomas in group 5 were observed in infected animals with little inflammation classified as grade 1. Intestinal metaplasia and adenocarcinomas were independently observed in animals infected with Hp, with no signs of spatial continuity. No adenocarcinomas were observed in groups 9 and 10.

Hp colonization

Hp were detected by culture in all MGs in groups 1, 3, 5 and 7 at week 30. Hp were also detected in 13 of 25 MGs in group 1, 18 of 24 in group 3, 15 of 26 in group 5, 24 of 25 in group 7, and 18 of 20 in group 9 at week 50. All MGs in groups 2, 4, 6, 8 and 10 were negative (Tables I and II).

Titres of anti-Hp antibody

Titres of anti-Hp antibodies of animals in groups 2, 4, 6, 8 and 10 were <10.0 AI. The average titre for MGs in the five groups was 2.8 (±0.2). The numbers of animals whose titres were <10.0 AI were 9/25, 4/24, 1/26, 1/25 and 0/20 in groups 1, 3, 5, 7 and 9, respectively. All MGs whose titres were >10.0 AI were negative for culture. Some MGs whose titres were >10.0 AI in group 5 had little inflammation evident macroscopically or microscopically. Titres of anti-Hp antibodies of tumour-bearing animals were 115.6 ± 34.3, 310.1 ± 63.8, 63.9 ± 24.0 and 208.2 ± 11.8 AI in groups 1, 3, 5 and 7, and these of tumour-free animals were 32.1 ± 16.0, 103.4 ± 19.0, 81.7 ± 9.7 and 123.5 ± 23.0 AI (average ± SE) in groups 1, 3, 5 and 7, respectively. Titres of anti-Hp antibodies of tumour-bearing animals were higher than those of tumour-free animals in groups 1, 3 and 7 (Figure 8).

Discussion

Colonization of the stomach mucosa by Hp has been reported in dogs (23,24), ferrets (25), monkeys (26) and mice (27–29). However, few studies on stomach carcinogenesis in animal models have been documented so far. We previously demonstrated that Hp infection enhances the development of pepsinogen-altered pyloric glands, a preneoplastic lesion of glandular stomach of BALB/c mice pretreated with MNU (30). In the reported study, glandular stomach cancers were not produced and other reports failed to confirm a relationship between Hp infection and stomach carcinogenesis (31–33). One hindrance may be the instability of Hp infection in animals. Hirayama et al. first established the Hp infection model in MGs and described development of chronic active gastritis, peptic ulcers and intestinal metaplasia (20). We subsequently established glandular stomach carcinogenesis models using MNNG and MNU in MGs (21). In a multi-step carcinogenesis protocol, we demonstrated that Hp infection enhanced glandular stomach carcinogenesis in MGs treated with MNU (22). In the present study, we proved that Hp infection also enhances glandular stomach carcinogenesis in MGs treated with MNNG. This implies that Hp infection enhances glandular stomach carcinogenesis in MGs, independent of the inducing carcinogen.
Table I. Histopathological changes of glandular stomachs in experiment I

<table>
<thead>
<tr>
<th>Group</th>
<th>1st treatment (mg/gerbil)</th>
<th>2nd treatment</th>
<th>MNNG intake (mg/gerbil)</th>
<th>No. of animals</th>
<th>Histology</th>
<th>Cancer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MNNG (300)</td>
<td>Hp</td>
<td>151.0</td>
<td>Initial</td>
<td>32</td>
<td>12 (44.4)*</td>
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<td></td>
<td></td>
<td>Br</td>
<td>155.3</td>
<td>Histologically examined</td>
<td>27</td>
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<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>2nd treatment</td>
<td>Hp</td>
<td>Br</td>
<td>53.6</td>
<td>19</td>
<td>14 chronic gastritis</td>
<td>0</td>
</tr>
<tr>
<td>2nd treatment</td>
<td>Br</td>
<td>Br</td>
<td>54.0</td>
<td>20</td>
<td>0 chronic gastritis</td>
<td>0</td>
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</table>

*Including five gerbils killed at week 30 for check of Hp infection.

*P < 0.01 versus group 2 by Fisher’s exact test; **P < 0.05 versus group 4 by Fisher’s exact test.

Hp, Helicobacter pylori (i.g.); Br, Brucella broth (i.g.); Well, well differentiated adenocarcinoma; Poor, poorly differentiated adenocarcinoma; Sig., signet-ring cell carcinoma.

Table II. Histopathological changes of glandular stomachs in experiment II

<table>
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<tr>
<th>Group</th>
<th>1st treatment</th>
<th>2nd treatment</th>
<th>MNNG intake (mg/gerbil)</th>
<th>No. of animals</th>
<th>Histology</th>
<th>Cancer (%)</th>
</tr>
</thead>
<tbody>
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<td>Hp</td>
<td>MNNG (100)</td>
<td>202.5</td>
<td>Initial</td>
<td>32</td>
<td>4 (14.8)*</td>
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<td>MNNG (100)</td>
<td>198.0</td>
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<td></td>
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<td>41.2</td>
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<tr>
<td>2nd treatment</td>
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<td>HP</td>
<td>54.3</td>
<td>22</td>
<td>0 chronic gastritis</td>
<td>0</td>
</tr>
</tbody>
</table>

*Including five gerbils killed at week 30 for check of Hp infection.

*P < 0.001 versus group 8 by Fisher’s exact test.

Hp, Helicobacter pylori (i.g.); Br, Brucella broth (i.g.); Well, well differentiated adenocarcinoma; Poor, poorly differentiated adenocarcinoma; Sig., signet-ring cell carcinoma.

MGs resemble humans in their susceptibility and response to Hp infection. It has already been reported that similar pathological changes occur in the glandular stomachs of man and MGs in response to Hp (20). Therefore, the MG model appears admirably suited for investigating the role of Hp in human stomach disorders. It is clear, however, that most persons infected with Hp will never develop stomach cancer and the bacterium cannot be the only causative factor (34). We previously demonstrated that the proportion of undifferentiated type lesions depends on the concentration of carcinogen given in mice (35). In the present study, animals treated with a high concentration of MNNG tended to develop undifferentiated adenocarcinomas. This suggests that the histological type of cancer depends on the genotoxic acting chemical carcinogen rather than the Hp infection. It has been proposed that chronic inflammation enhances cell proliferation (16,36,37), which
may enhance carcinogenesis by increasing the turnover of initiated cells.

In this study, we observed signet-ring cell carcinomas, as well as poorly differentiated adenocarcinomas and well differentiated adenocarcinomas. This variety of induced stomach cancers, as well as response to Hp infection like in man suggests advantages of this model for research on stomach.
Ca. (1) groups 1, 3 and 7 by Mann–Whitney's test (Fig. 8), tumour-bearing animals; Ca.(–), tumour-free animals.

Fig. 7. Histological sections of gastric mucosa from animals at week 50. A signet ring cell carcinoma in a group 1 gerbil (AB-PAS, ×150).

Fig. 8. Titres of anti-Hp antibodies in experiments I and II. Titres of tumour-bearing animals are higher than those of tumour-free animals in groups 1, 3 and 7 by Mann–Whitney's U-test (*P < 0.05; **P < 0.01). Ca.(+), tumour-bearing animals; Ca.(–), tumour-free animals.

carcinogenesis in human. Until recently, it has been considered that Hp infection causes atrophic gastritis followed by development of intestinal metaplasia and well differentiated adenocarcinomas (5). However, a recent meta-analysis indicated that Hp infection is equally associated with intestinal and diffuse types of gastric cancer (38), in agreement with our findings. Intestinal metaplasia in humans has been considered to be a preneoplastic change for well differentiated adenocarcinomas (39,40), but in the present experiment, no relationship between intestinal metaplasia and glandular stomach cancers induced by Hp infection and MNNG administration in MGs was found. The data in this work are also consistent with the conclusion from our previous studies using a rat model (41) or human materials (42,43) that intestinal metaplasia is not a preneoplastic change of any major relevance to gastric neoplasia.

The observed discrepancies between positive rates for antibodies, culture, immunohistochemical examination and inflammation states in histology were presumably due to methodological limitations. Elevation of anti-Hp antibodies means that animals were at some time infected with Hp, but are not necessarily so at the time of sampling. It is known that the titres of anti-Hp antibodies are still high ~6 months after eradication in human cases (44), so false positive cases are unavoidable. Positive culture for Hp confirms the presence of Hp in tissue samples, but does not give any clue to the level of infection as a whole. Immunohistochemical examination can give a more precise assessment of the bacterial population, while microscopic inflammation indicates the host response to Hp and is under the influence of host immunity.

One interesting point in this model is the reason for the persistent Hp infection in the stomachs of MGs, not found in mice and rats. The organism obviously prefers to colonize the layer of surface mucous cell type mucins in the human stomach (45). The decreased rate of Hp infection with ageing (46) and with stomach mucosal damage by MNNG in MGs as noted in this study, and increase in Hp infection related to stomach mucosal damage by MNU or a salty diet in mice (30) imply important roles for mucous conditions of the stomach mucosa for Hp infection. It is essential to clarify this point to develop effective prevention strategies.

To determine the pathogenic role of Hp, it is necessary to investigate the interaction between parasite and host. Important roles have been demonstrated for cagA and vacA genes in the pathogenicity of Hp (47–49). Since Tomb et al. elucidated the complete genome sequence of the Hp in 1997 (50), it is likely that a fuller understanding will be generated in the near future. Concerning the host interaction, it was demonstrated that T helper 1 cellular immune responses contribute to Helicobacter-associated gastritis in mice (51) and man (52), and D’Elios et al. showed that Hp-specific T helper 1 effectors may play a role in peptic ulcers in humans (53). In our study, although overlap of titre levels between groups was observed, the titres of anti-Hp antibodies of the tumour-bearing animals were higher than in tumour-free animals treated in the same manner. It is reported that immunosuppression is observed during carcinogen-induced tumour development (54). So we think it appropriate that titres were evaluated within each group, but not in general, because different treatments may result in a different immune status. The data imply that humoral immunity, which may mean T helper 2 response (55,56), is dominant with regard to neoplasia, in contrast to the T helper 1 dominance in sufferers from peptic ulcers. In a murine B-cell leukaemia/lymphoma model, susceptible hosts developed a T helper 2 dominant response, whereas resistant hosts developed a T helper 1 dominant response (57).

Watanabe et al. have reported the development of mainly well differentiated adenocarcinomas in the stomachs of MGs infected with Hp without chemical carcinogen (58). Different from our study, they use a clinically isolated strain derived from a patient suffering from peptic ulcers. Some Hp strains may be more related to neoplastic development than others (15), although infection is equally associated with intestinal and diffuse types of gastric cancer (38). We previously demonstrated remission of lesions arising in the gastric stump of rats when bile reflux was interrupted (59,60). This result indicated that the lesions which were generally considered as neoplasms
are not always real malignancies. Therefore, it is necessary to check the effects of eradication on lesions that arise in the glandular stomach of MGs infected with Hp, because tissue damage is so severe that ectopic proliferation, which is one criterion of neoplasia, cannot be applied in this case. True neoplastic lesions would be expected to continue growing independently after eradication, while reactive lesions would go into remission.

It is very interesting in the present study that 100 p.p.m. MNNG administration for 30 weeks itself eradicated the Hp in about half the infected animals so that the enhancing effect on glandular stomach carcinogenesis was reduced. All animals were positive for Hp culture at week 30 and all but one had high titres of anti-Hp antibodies at week 50, confirming past Hp infection. Thus, eradication of Hp is likely to be useful for cancer prevention, as well as being a therapy for peptic ulcers (61), stomach mucosa-associated lymphoid tissue lymphomas (62,63) and dyspepsia (64,65). Moreover, compared with animals treated with Hp followed by 20 p.p.m. MNNG administration, the enhancing effect of Hp infection may be more important than the dose of MNNG.

Using the present carcinogenesis model, further eradication, sequential histopathological and molecular biological examinations should allow detailed assessment of the risk of Hp infection in terms of gastric neoplasia and elucidation of the mechanisms of its adverse influence.

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