Helicobacter pylori-infected animal models are extremely suitable for the investigation of gastric carcinogenesis

Masaaki Kodama, Kazunari Murakami, Ryugo Sato, Tadayoshi Okimoto, Akira Nishizono, Toshio Fujioka

INTRODUCTION
Gastric cancer is one of the main causes of cancer-related mortality, especially, in East Asia. To clarify the mechanism of gastric cancer development, many experimental models have been used. However, almost all experimental animals, that showed spontaneous gastric cancer were very rare; therefore several animal models were established using chemical carcinogens, such as N-methyl-N-nitrosourea (MNU) [2,3] and N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) [4,8], which showed a high rate of gastric cancer development, especially in the antrum.

Since Warren and Marshall [8] revealed the microorganism which inhabits the stomach, Helicobacter pylori (H pylori) was considered as the major factor of many kind of gastrodudodenal diseases, such as acute gastritis [9,15], chronic atrophic gastritis [9,12,14], intestinal metaplasia [19,20], peptic ulcer [14], mucosal associated lymphoid tissue lymphoma [8], gastric cancer [9,12,13], and others [11,19].

Previously, a large number of epidemiological studies indicated that H pylori infection has a close relation with gastric cancer [16-18]. Therefore, the International Agency for Research on Cancer (IARC) conference of the World Health Organization (WHO) defined H pylori as a definite carcinogen (Group I) to the human stomach based on three prospective case-control studies [15-17] reported in 1991 [20].

However, the mechanisms by which H pylori infection develop gastric cancer are not defined in detail. In further studies, attempts have been made to reveal the possible mechanisms by which H pylori contributes to the development of gastric carcinoma and many researchers have developed animal models of infection using Helicobacter species.

Previously, a large number of animal experimental models have been developed to define the association between H pylori infection and gastrodudodenal disease, such as piglet [21], beagle dog [22], mice [23], rhesus monkey [24], Japanese monkey (Macaca fuscata) [25,26,27], Mongolian gerbil [28], and others. In the beginning of the development of experimental models, only a few models had long periods of infection.

We have reported the results of a 5-year study on H pylori infection using Japanese monkeys (Macaca fuscata) [27].

Abstract
Although various animal models have been developed to clarify gastric carcinogenesis, apparent mechanism of gastric cancer was not clarified in recent years. Since the recognition of the pathogenicity of Helicobacter pylori (H pylori), several animal models with H pylori infection have been developed to confirm the association between H pylori and gastric cancer. Nonhuman primate and rodent models were suitable for this study. Japanese monkey model revealed atrophic gastritis and p53 mutation after long-term infection of H pylori. Mongolian gerbil model showed the development of gastric carcinoma with H pylori infection alone, as well as with combination of chemical carcinogens, such as N-methyl-N-nitrosourea and N-methyl-N-nitro-N'-nitrosoguanidine. The histopathological changes of these animal models after H pylori inoculation are closely similar to those in human beings with H pylori infection. Eradication therapy attenuated the development of gastric cancer in H pylori-infected Mongolian gerbil. Although several features of animal models differ from those seen in human beings, these experimental models provide a starting point for further studies to clarify the mechanism of gastric carcinogenesis as a result of H pylori infection and assist the planning of eradication therapy to prevent gastric carcinoma.

Key words: Helicobacter pylori; Gastric carcinoma; Animal model; Japanese monkey; Mongolian gerbil

Kodama M, Murakami K, Sato R, Okimoto T, Nishizono A, Fujioka T. Helicobacter pylori infected animal models are extremely suitable for the investigation of gastric carcinogenesis. World J Gastroenterol 2005; 11(45): 7063-7071

http://www.wjgnet.com/1007-9327/11/7063.asp
and have obtained the findings that advance gastric mucosal atrophy, increase proliferation and mutation of p53 in gastric epithelial cells[20,30].

Several experiments, which demonstrated that chronic H pylori infection models of Mongolian gerbils developed gastric carcinoma, were conducted[31–33]. In these experiments, the animals were mainly divided into two groups: one group was infected with H pylori alone and the group was given a known carcinogen such as MNU and MNNG in addition to persistent H pylori infection. The results of these experiments revealed that animals in different groups developed different histopathological types of gastric carcinoma. These results will be very useful to elucidate the mechanism of gastric carcinogenesis due to H pylori infection.

GASTRIC CANCER AND JAPANESE MONKEY

The nonhuman primate animals are useful to clarify the relationship between H pylori and gastric diseases. Their stomachs are similar to those of human beings anatomically, physiologically, and dietary, compared with rodent animals. They have 10–20 years of long life span, which enables long-term follow-up with endoscopy and repeated histological examinations of the stomach using biopsy or endoscopic resected specimens. Several primate animals have been reported to be successful in experimental transmission of H pylori in chimpanzees (Pan troglodytes)[34], and species of macaques: rhesus monkey (M. mulatta)[35], cynomolgus monkey (M. fascicularis)[36], and Japanese monkey (M. fuscata)[37]. In these animals, some kinds of Macaque species are available for a wide variety of research field. We have established the Japanese monkey model with H pylori infection. This experimental model is very useful and a promising nonhuman primate mode[20,21,27].

The methods of development of this monkey model are described briefly. The bacterial strains used were H pylori MCO 88155, MCO 88099, MCO 88142, and MCO 88156, isolated from two patients with duodenal ulcers and two with gastric ulcers. The colonies were suspended in 5 mL of sterile saline, and the bacterial concentration was adjusted to 10^9 CFU/mL. These were resuspended in 8 mL of sterile saline, and 5 mL of the final resuspension was used in each monkey. The animals were given ampicillin orally to eradicate spiral bacteria other than H pylori. After treatment with ampicillin, spiral bacteria were not found in any of the stomachs. The monkeys were sprayed with 5 mL of a mixed suspension of four bacterial strains endoscopically around their antrum. The gastric mucosa was examined endoscopically, and endoscopic mucosal resection was performed repeatedly during 6 years of observation.

One week after inoculation, all infected monkeys showed endoscopic acute gastritis accompanied by marked erythema and edema. These findings were consistent with the acute gastric mucosal lesion observed in the human stomach. Infection of H pylori was recognized by culture, the rapid urease test, histology, and the elevation of H pylori-specific IgG in plasma. In the early phase of infection, infiltration of mononuclear and polymorphonuclear leukocytes were marked in the edematous lamina propria and superficial erosions were evident. After 3 mo of inoculation, infiltration of mononuclear cells and plasma cells were predominant in the lamina propria layer. However, no superficial erosions and atrophic changes were observed.

In the infected group, the gastritis score which was evaluated by a scoring system based on the method of Rauws et al[37]. were markedly increased in the antral mucosa 1 wk after inoculation (P<0.001). The score then gradually decreased throughout the whole investigation period, but remained significantly higher (P<0.01) than that of the control group.

Six months after inoculation, the pyloric glandular height was apparently lower in the infected animals than in controls. Furthermore, the atrophic change advanced gradually throughout the 5-year observation period[27]. Endoscopically, according to the endoscopic-atrophic-border scale described by Kimura and Takemoto[36], gastric atrophy also gradually advanced for more than 3 years. These findings indicated evidently that H pylori infection caused atrophic gastritis in the Japanese monkey model. Cell proliferation activity, which was revealed with immunohistochemical detection of Ki-67 in the antral mucosa of infected animals, was significantly accelerated throughout the entire observation period (Figure 1). Immunohistochemical detection of p53 and point mutation of p53 was exhibited in the gastric mucosa[20,30] of this model. Genetic alterations in exons 5–8 of the p53 gene were uncommon in the H pylori-uninfected monkeys, whereas a higher prevalence of missense mutations in the p53 gene appeared in association with H pylori infection (Table 1). The number of mutations in the p53 gene increased as the gastric atrophy score increased, which depends on the duration of H pylori infection[28]. These findings of Japanese monkey model may explain the potential mechanism for the causal role of H pylori in the chain of events leading to gastric carcinoma. This monkey model facilitates investigation of the correlation between the long-term sequence of H pylori infection and gradual gastric mucosal change. Although many pathophysiological changes were seen in H pylori-infected gastric mucosa, this Japanese monkey model did not show the development of gastric carcinoma. In their long life span, which is similar to human beings, further continuous infection may be needed to the more dramatic histological change.

DEVELOPMENT OF THE RODENT MODEL

Several rodent models were established for examining the etiologic feature of Helicobacter species infection, such as mice[29,31], rat[30], and Mongolian gerbil[32]. Compared with nonhuman primate models, rodent models are treated easily, and are economical.

Marchetti et al[30] reported the several clinical isolates colonized the stomach of SPF conditioned mice (CD1 mice) and Balb/c mice; however, colonization was very...
In our laboratory, 5-wk-old male Mongolian gerbils weighing 30-40 g (Seiwa Experimental Animals Co. Ltd., Fukuoka, Japan) were used. They were inoculated with C. jejuni possessing the cagA gene and expressing vacuolating cytotoxins. The fish were housed in a separate room with sterile bedding. The animals were monitored daily for any signs of illness or adverse reactions.

Table 1 Duration of *H. pylori* infection and number of point mutations in exon 5-8 of the p53 gene

| Monkey | Duration of *H. pylori* infection (yr) | Number of nucleotide substitutions in p53 (amino acid) | Atrophy score | Intensity of p53 immunostaining |
|--------|--------------------------------------|-------------------------------------------------------|--------------|-------------------------------|
| A      | Ex 5                                 | 2 (1)                                                 | 4            | +                             |
| B      | Ex 6                                 | 3 (2)                                                 | 4            | +                             |
| C      | Ex 7                                 | 5 (3)                                                 | 6            | ++                            |
| D      | Ex 8                                 | 4 (2)                                                 | 5            | +                             |

1. The atrophy score was calculated as the sum of the histological evaluations of five gastric specimens according to Updated Sydney System. 2. The intensity of p53 immunostaining was classified into four grades: -, no staining; +, mild staining; ++, moderate staining; ++++, intense staining.

In our laboratory, 5-wk-old male Mongolian gerbils weighing 30-40 g (Seiwa Experimental Animals Co. Ltd., Fukuoka, Japan) were used. They were inoculated with *C. jejuni* possessing the cagA gene and expressing vacuolating cytotoxins. The fish were housed in a separate room with sterile bedding. The animals were monitored daily for any signs of illness or adverse reactions.
MI, USA) with 10% horse serum for 24 h. Inoculum size was adjusted with sterile saline to produce the optical density of McFarland 4 at 540 nm. Mongolian gerbils were housed five per cage, starved for 24 h, and then fed with chow (Oriental Yeast Co., Tokyo, Japan) and water ad libitum beginning 12 h after H pylori inoculation. On the day of infection, the Mongolian gerbils were challenged orally with vehicle or 10⁹ CFU H pylori in 1.0 mL of brucella broth with 10% horse serum. The spiral bacteria were observed in the mucus and gastric pits of all inoculated animals from 1 mo after inoculation throughout the whole observation period. However, nearly half of the animals had barely detectable H pylori in the stomach by bacterial culture. The bacterial counts from the stomachs of gerbils 1 and 6 mo after H pylori inoculation were 25 and 410 CFU/10 mg of gastric tissue, respectively.⁴⁸ These levels of colonized bacteria were nearly 1/10 to 1/100 than those of human being and monkey.

Mongolian gerbils with H pylori infection showed irregularly thickened gastric walls and sploty hemorrhages and erosions macroscopically, 1 year after inoculation. A severe infiltration of polymorphonuclear and mononuclear cells was seen in the lamina propria and mononuclear cells infiltration with lymphoid follicle in the submucosa, 1 mo after H pylori inoculation (Figure 2A). Erosion of the gastric mucosa appeared soon after inoculation, whereas gastric ulcers, gastritis cystica profunda (Figure 2B), and atrophy with goblet cell metaplasia (Figures 2C and D) occurred between 3 and 6 mo after inoculation.⁴⁸ Moreover, Suzuki et al⁴⁹ reported that H pylori inoculation induced neutrophil followed by an increase in the level of lipid peroxidation and activated glutathione (antioxidant) turnover. These sequential changes of histological changes in gastric mucosa were quite similar to those observed in human beings. Therefore, Mongolian gerbil model may be useful to study the relationship between H pylori infection and gastric lesions, which include gastric malignancy.

GASTRIC CANCER AND MONGOLIAN GERBIL MODEL

Mongolian gerbils have also been induced by the development of gastric carcinoma with chemical infection and early events in gastric carcinogenesis. In addition, the results of several experimental studies have confirmed that administration of MNNG or MNU to Mongolian gerbils with chronic H pylori infection enhanced the development of different histopathological types of gastric carcinoma (Table 2)⁵¹-⁵³. Sugiyama et al⁵⁴ reported the development of carcinoma in the Mongolian gerbils evaluated at 40 wk after an experiment in which 7-wk-old animals were inoculated with H pylori (ATCC43504) and given 10 or 30 ppm MNU before or after inoculation. In this report, only the groups of the animals, which were administered with both H pylori and MNU, developed gastric cancers; more specifically, they developed different types of adenocarcinoma, such as well-differentiated, poorly differentiated, and signet ring cell carcinoma. These interesting experimental results support the results so far obtained in largescale epidemiological investigations.⁵⁵

The group inoculated with H pylori after being given MNU showed a distinctive initiation-promotion effect, whereas the group to which MNU was given after inoculation with H pylori appeared to demonstrate the simultaneous action of these two factors, with H pylori acting as a coinitiator. No gastric carcinoma was found within 40 wk of H pylori infection alone.

Tokieda et al⁵⁶ conducted a study in which 5-wk-old Mongolian gerbils were inoculated with H pylori (ATCC43504) and orally given MNNG at 50 g/mL for 20 wk for comparison against animals administered with MNNG alone. At the 52nd wk after initiation, the group treated with MNNG and H pylori developed gastric carcinoma at a significantly higher frequency than the group treated with MNNG alone. In addition, cell proliferation was revealed to be markedly accelerated in those animals infected with H pylori with evaluation using a labeling index of 5-bromo-2-deoxyuridine. This result suggests the possibility of explaining the link between H pylori infection and early events in gastric carcinogenesis.

One of the interests in this study is that administration of MNNG reduced the infection rate of H pylori with the lapse of time, due to the likelihood of MNNG showing low-level (200 μg/mL) antibacterial activity against H pylori⁵⁷. It is also of interest that H pylori-free animals did not develop gastric carcinoma even with...
infection. Although it has been isolated from patients infected Mongolian gerbils with MNNG infection, resulting in infection and gastric cancer was not indicated. It is interesting that the two studies that reported MNNG administration eradicates it is persistent HP positive carcinogenic effects in the stomach. The results may probably depend on the relationship between chemical carcinogenic risk in the Mongolian gerbils, direct eradication of HP, and the establishment of gastric adenocarcinoma and carcinoid were developed in these strains. Sequential histopathological changes leading to carcinogenesis of the gastric mucosa were found to be common to the two studies, and very closely resembled the histopathological changes in human gastric mucosa caused by MNNG administration. This result indicated a stronger carcinogenic role of *H. pylori* infection. Although it has been reported by Sugiyama et al. that *H. pylori* can persistently colonize the stomach of MNUTreated Mongolian gerbils, it is interesting that the two studies report that MNNG administration eradicates *H. pylori* infection, resulting in a reduction of its carcinogenic effects in the stomach. In *H. pylori*-infected Mongolian gerbils with MNNG administration, duodenogastric reflux due to surgical procedure might attenuate the effect of *H. pylori* on gastric tumorigenesis. Because of our study, which indicated that bile reflux might lead to *H. pylori* eradication, their results may probably depend on the *H. pylori* eradication.

### Carcinogenicity of *H. pylori* Infection Alone

Although these studies showed marked increase of the chemical carcinogenic risk in the Mongolian gerbils, direct relationship between *H. pylori* and gastric carcinogenesis was not indicated.

Two experimental studies attempted to confirm prior epidemiological studies that have demonstrated an association between *H. pylori* infection and gastric carcinogenesis in human beings using Mongolian gerbils chronically infected with this bacterium. Both studies confirmed gastric carcinogenesis resulting from *H. pylori* infection alone, and were the first papers to fulfill Koch’s postulates concerning *H. pylori* infection and gastric carcinoma.

Watanabe et al. used *H. pylori* isolated from patients with gastric ulcer (TN2GF4), and Honda et al. used ATCC43504 type strain, both of which were inoculated into 5-wk-old SPF Mongolian gerbils. The results showed that 37% (10 out of 27) of the animals in the former study developed well-differentiated adenocarcinoma at 62 wk after inoculation, whereas 40% (2 out of 5) of the animals in the latter study developed well-differentiated adenocarcinoma at 72 wk after inoculation (Figure 3). Both of these strains contained *cagA* and produced vacuolating cytotoxins. Sequential histopathological changes leading to carcinogenesis of the gastric mucosa were found to be common to the two studies, and very closely resembled the histopathological changes in human gastric mucosa caused by *H. pylori* infection.

Hirayama et al. reported that poorly differentiated adenocarcinoma and carcinoid were developed in

---

**Table 2** Gastric carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils

| Author            | Year | Strain            | Study design (ppm)                     | Incidence of cancer (%) | Duration of experiment (wk) |
|-------------------|------|-------------------|----------------------------------------|-------------------------|----------------------------|
| Sugiyama et al.   | 1998 | ATCC43504         | HP → MNU (10)                          | 7/19 (36.8)             | 40                         |
|                   |      |                   | HP alone                               | 0/20 (0)                | 40                         |
|                   |      |                   | MNU (30) → HP                          | 6/18 (33.3)             | 40                         |
|                   |      |                   | MNU alone                              | 0/74 (0)                | 40                         |
| Tokieda et al.    | 1999 | ATCC43504         | HP → MNN (50)                          | 5/17 (29.4)             | 52                         |
|                   |      |                   | persistent HP positive Br → MNN (50)   | 5/8 (62.5)              | 52                         |
|                   |      |                   | HP eradicated                          | 0/9 (0)                 | 52                         |
| Shimizu et al.    | 1999 | ATCC43504         | MNNG (300) → HP                        | 12/27 (44.4)            | 50                         |
|                   |      |                   | MNNG (300) → Br                        | 1/19 (5.3)              | 50                         |
|                   |      |                   | MNNG (60) → HP                         | 6/25 (24.0)             | 50                         |
|                   |      |                   | MNNG (60) → Br                         | 0/20 (0)                | 50                         |
|                   |      |                   | HP → MNNG (100)                        | 4/27 (14.8)             | 50                         |
|                   |      |                   | Br → MNNG (50)                         | 3/18 (16.7)             | 50                         |
|                   |      |                   | HP → MNNG (20)                         | 15/25 (60)              | 50                         |
|                   |      |                   | HP eradicated Br → MNNG (50)           | 5/22 (13.6)             | 50                         |
|                   |      |                   | persistent HP positive HP eradicated    | 0/9 (0)                 | 52                         |

**Table 3** Gastric carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils

| Author            | Year | Strain         | cagA gene | Vacuolating cytotoxin | Incidence of cancer (%) | Duration of experiment (wk) | Histological type of carcinoma |
|-------------------|------|----------------|-----------|-----------------------|-------------------------|----------------------------|-------------------------------|
| Watanabe et al.   | 1998 | TN2GF4         | +         | +                     | 10/27 (37)              | 62                         | Well differentiated adenocarcinoma |
| Honda et al.      | 1998 | ATCC43504      | +         | +                     | 2/5 (40)                | 72                         | Well differentiated adenocarcinoma |
| Hirayama et al.   | 1999 | ATCC43504      | +         | +                     | 1/56 (1.8)              | 64                         | Poorly differentiated adenocarcinoma |
| Ogura et al.      | 2000 | TN2           | +         | +                     | 1/23 (4)                | 62                         | Well differentiated adenocarcinoma |
| Zheng et al.      | 2004 | ATCC43504      | +         | +                     | 3/17 (18)               | 84                         | Well differentiated adenocarcinoma |
|                   |      | *H. pylori* 161 | +         | +                     |                         |                            |                                |

1. *H. pylori* isolated from patient with gastric ulcer; 2. Type of strains; 3. *H. pylori* isolated from patient with gastric adenocarcinoma.
Mongolian gerbils model with *H pylori* (ATCC43504 type strain) infection alone. Zheng et al.\(^5\) reported that Mongolian gerbils models, which were infected with *H pylori* (ATCC43504) and *H pylori* 161 (isolated from a Chinese patient with gastric adenocarcinoma) showed the development of well-differentiated adenocarcinoma (Table 3). Ogura et al.\(^5\) reported the development of well-differentiated gastric cancer in wild type (TN2) and isogenic mutant of *vacA* (TN2Δ*vacA*) of Mongolian gerbil.

Mongolian gerbils\(^6\) model also showed the development of gastric carcinoid.\(^5\)\(^7\)\(^9\)\(^8\)\(^6\)\(^4\)\(^1\) In our laboratory, ECL cell tumors with marked atrophic gastritis and with hypergastrinemia were observed in the fundic gland area of infected Mongolian gerbils, 24 mo after inoculation (Figure 4); in contrast, adenocarcinoma developed in pyloric gland area. Histopathological findings of the entire observation period in Mongolian gerbils after *H pylori* inoculation are summarized in Table 4.

Ogura et al.\(^5\) discussed the virulence factors of *H pylori* in Mongolian gerbils. Experimental gastric cancer derived in Mongolian gerbils with wild type of *H pylori* and *vacA* mutant infection, whereas *cagE* mutant induced far milder change of gastritis and induced no gastric cancer, which indicates the essential role of *cagPAI* in the gastric diseases with *H pylori* infection.

| Table 4 Gastric carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils |
|-----------------------------------------------|
| Histopathological findings                  | Mo  |
| ---------------------------------------------|-----|
|                                             | 6   | 12 | 18 | 24 |
| Gastritis                                    | 5/5 | 4/4 | 5/5 | 10/10 |
| Gastric ulcer                                | 4/5 | 3/4 | 5/5 | 10/10 |
| Atrophy                                      | 4/5 | 4/4 | 5/5 | 10/10 |
| Intestinal metaplasia                        | 2/5 | 3/4 | 5/5 | 10/10 |
| Dysplasia                                    | 0/5 | 2/4 | 4/5 | 10/10 |
| Gastric cancer                               | 0/5 | 0/4 | 2/5 | 5/10  |
| Gastric carcinoid                            | 0/5 | 0/4 | 0/5 | 5/10  |

Data represent positive case/control Uninfected control, animals (*n* = 5 each) showed no abnormal findings.

| Table 5 Relation between p53 and *H pylori* and gastric mucosal change (tentative opinion) |
|-----------------------------------------------------------------------------------------|
| Condition                                                                 | Human | Japanese monkey | Mongolian gerbil |
| *H pylori* and p53 overexpression (histology)                                          | ++    | ++              | ++              |
| *H pylori* and p53 point mutation                                                     | ++    | ++              | -               |
| *H pylori* and atrophic gastritis                                                     | ++    | ++              | ++              |
| *H pylori* and intestinal metaplasia                                                  | ++    | -               | ++              |
| *H pylori* and gastric cancer                                                        | +\(^1\) | -               | ++              |

\(^1\)The p53 overexpression was observed only in gastric cancer; ++, strong evidence; +, weak evidence; -, no evidence

### PREVENTION OF GASTRIC CARCINOMA BY ERADICATION OF *H PYLORI*

Shimizu et al.\(^6\) reported that the incidence of adenocarcinomas in MNU-administered Mongolian gerbils with *H pylori* infection (15 out of 23) was significantly higher than in MNU-administered Mongolian gerbils that underwent *H pylori* eradication (5 out of 24). Their results suggest that *H pylori* eradication may prevent gastric carcinogenesis, and Mongolian gerbil’s models have also been useful to study the prevention of gastric carcinogenesis in human beings.

### DIFFERENCES BETWEEN ANIMAL MODELS AND HUMAN BEINGS

Although the Japanese monkey model and Mongolian gerbil model showed the similar change of human stomach that was infected with *H pylori*, several features of animal models differ from those seen in human beings. In Japanese monkey model intestinal metaplasia was not seen during the whole observation period. Severe gastritis and lymphoid follicular hyperplasia in the submucosal layer and gastritis cystica profunda, which are seen in Mongolian gerbils, are not observed in human gastric mucosa.

Table 5 shows our tentative opinion on p53 and *H pylori* infection in animal model and human beings. Although no gastric carcinoma developed in Japanese monkey model, Mongolian gerbil model showed gastric carcinoma resulting from *H pylori* infection alone. In human and Japanese monkey, both p53 immunostaining\(^5\)\(^9\)\(^6\)\(^8\)\(^4\) and point mutations\(^8\)\(^6\)\(^4\) were observed in *H pylori* infection. In
Mongolian gerbil, the p53 immunostaining was detected in gastric cancer but not in atrophic gastritis; moreover, there were no p53 mutations in exons 5 to 8 in infected gastric mucosa\cite{66}.

Suzuki et al\cite{66} reported that Mongolian gerbil model showed significant attenuation of apoptosis and promotion of cell proliferation than those seen in mice model with \textit{H. pylori} inoculation. Crabtree et al\cite{69}, also described the differences of mucosal cytokine response between Mongolian gerbils and mice, and gender differences in the magnitude of cytokine response to \textit{H. pylori}. The differences of features between the species suggested that the pathogens of gastric diseases does not associate only with \textit{H. pylori} and may reflect in part other host factors.

Sonic hedgehog (Shh) is an important endometrial morphogenetic signal during the development of the vertebrate gut. Shh controls gastrointestinal patterning in general and gastric gland formation in particular. Suzuki et al\cite{69}, reported that the long-term colonization of \textit{H. pylori} led to attenuation of Shh expression. Loss of Shh expression correlated with the loss of parietal cells, disturbed maturation of the mucous neck cell-zymogenic cell lineage. van den Brink et al\cite{69}, described the loss of Shh expression in the intestinal metaplasia of the human stomach. Loss of Shh expression not only in intestinal metaplasia, but also in the tissue of \textit{H. pylori}-induced fundic gland atrophy is important for considering the possible link to preneoplastic lesion formation\cite{69}.

**QUITE A NEW CONCEPT OF GASTRIC CANCER ORIGIN**

In 2004, Houghton et al\cite{70}, reported the innovative idea of gastric cancer origin with the usage of \textit{H. felis} C57BL/6 mouse model. Previously, tissue stem cells have been recognized as the origin of carcinoma. However, their study showed that bone marrow-derived cells (BMDCs) might also represent a potential source of carcinoma. van den Brink et al\cite{69}, described the loss of Shh expression in the intestinal metaplasia of the human stomach. Loss of Shh expression not only in intestinal metaplasia, but also in the tissue of \textit{H. pylori}-induced fundic gland atrophy is important for considering the possible link to preneoplastic lesion formation\cite{69}.

**REFERENCES**

1. Cui G, Qvigstad G, Falkmer S, Sandvik AK, Kawase S, Waldum HL. Spontaneous ECLomas in cotton rats (Sigmodon hispidus): tumours occurring in hypochloric/ hypergastrinaemic animals with normal parietal cells. Carcinogenesis 2000; 21: 23-27.
2. Fort L, Taper HS, Brucher JM. Gastric carcinogenesis in rat induced by methyl nitrosourea (MNU). Morphology, and histochemistry of nucleases. Z Krebsforsch Klin Onkol Cancer Res Clin Oncol 1981; 81: 51-62.
3. Fujita M, Taguchi T, Takami M, Usugane M, Takahashi A. Lung metastasis of canine gastric adenocarcinoma induced by N-methyl-N’-nitro-N-nitroguanidine. Gan 1975; 66: 107-108.
4. Kartasheva LA, Bykorez AL. Induction of stomach tumors in rats by N-methyl-N-nitroso-N-nitroguanidine. Vopr Onkol 1975; 21: 50-55.
5. Koestner AW, Rueckel FA, Koestner A. Morphology and pathogenesis of tumors of the thymus and stomach in Sprague-Dawley rats following intragastric administration of methyl nitrosourea (MNU). Int J Cancer 1977; 20: 418-426.
6. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; I: 1311-1315.
7. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfill Koch’s postulates for pyloric Campylobacter. Med J Aust 1985; 142: 436-439.
8. Morris A, Nicholson G. Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastroenterol 1987; 82: 192-199.
9. Fujioka T, Shuto R, Kodama R, Fujiyama K, Kubota T, Murakami K, Perparim K, Nasu M. Experimental model for chronic gastritis with Helicobacter pylori: long term follow-up study in \textit{H. pylori}-infected Japanese macaques. Eur J Gastroenterol Hepatol 1993; 5 (suppl 1): S73-S78.
10. Kuipers EJ, Uytterlinde AM, Peña AS, Roosendaal R, Pals G, Nels GF, Festen HP, Meuwissen SG. Long-term sequelae of \textit{Helicobacter pylori} gastritis. Lancet 1995; 345: 1525-1528.
11. Sakaki N, Momma K, Egawa N, Yamada Y, Kan T, Ishiwata J. The influence of \textit{Helicobacter pylori} infection on the progression of gastric mucosal atrophy and occurrence of gastric cancer. Eur J Gastroenterol Hepatol 1995; 7 Suppl 1: S59-S62.
12. Schubert TT, Bolognina SD, Nensey Y, Schubert AB, Mascha EJ, Ma CK. Ulcer risk factors: interactions between \textit{Helicobacter pylori} infection, nonsteroidal use, and age. Am J Med 1993; 94: 413-418.
13. Maaroos HI, Kekki M, Vorobjova T, Salupere V, Sipponen P. Risk of recurrence of gastric ulcer, chronic gastritis, and grade of \textit{Helicobacter pylori} colonization. A long-term follow-up study.
14. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 1993; 342: 575-577
15. Forman D, Newell DG, Fuehrer F, Yarnall JW, Stacey AR, Wald N, Sitas F. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BMJ 1991; 302: 130-1305
16. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991; 325: 1127-1131
17. Nomura A, Steemernmann GN, Chyoo PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991; 325: 1123-1136
18. An international association between Helicobacter pylori infection and gastric cancer. The EUROGAST Study Group. Lancet 1993; 341: 1359-1362
19. Gasbarrini A, Franceschi F, Tartaglione R, Landolfi R, Pola P, Gasbarrini G. Regression of autoimmune thymocytopenia after eradication of Helicobacter pylori. Lancet 1998; 351: 878
20. Sato R, Murakami K, Watanabe K, Okimoto T, Miyajima H, Ogata M, Ohtsuka E, Kodama M, Saburi Y, Fujioka T, Nasu M. Effect of Helicobacter pylori eradication on platelet recovery in patients with chronic idiopathic thymocytopenic purpura. Arch Intern Med 2004; 164: 1904-1907
21. International Agency for Research on Cancer. Infection with Helicobacter pylori. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1994; 61: 218-220
22. Krakowka S, Morgan DR, Kraft WG, Leunck RD. Establishment of gastric Campylobacter pylori infection in the neonatal gnotobiotic piglet. Infect Immun 1987; 55: 2789-2796
23. Radin MJ, Eaton KA, Krakowka S, Morgan DR, Lee A, Otto G, Fox J. Helicobacter pylori gastric infection in gnotobiotic beagle dogs. Infect Immun 1990; 58: 2606-2612
24. Karita M, Kouchiyama T, Okita K, Nakazawa T. New small animal model for human gastric Helicobacter pylori infection: success in both nude and euthymic mice. Am J Gastroenterol 1991; 86: 1596-1603
25. Euler AR, Zurenko GE, Moe JB, Ulrich RG, Yagi Y. Evaluation of two monkey species (Macaca mulatta and Macaca fascicularis) as possible models for human Helicobacter pylori disease. J Clin Microbiol 1990; 28: 2285-2290
26. Shudo R, Fujioka T, Kubota T, Nasu M. Experimental gastritis induced by Helicobacter pylori in Japanese monkeys. Infect Immun 1993; 61: 933-939
27. Fujioka T, Kodama R, Honda S, Guei-Hua G, Nishizono A, Nasu M. Long-term sequelae of experimental gastritis with Helicobacter pylori: a 5-year follow-up study. J Clin Gastroenterol 1997; 25 Suppl 1: S8-S12
28. Yokota K, Kurebayashi Y, Takayama Y, Hayashi S, Isogai H, Isogai E, Inai K, Yabana T, Yachi A, Oguma K. Colonization of Helicobacter pylori in the gastric mucosa of Mongolian gerbils. Microbiol Immunol 1991; 35: 475-480
29. Kodama M, Fujioka T, Kodama R, Takahashi K, Kubota T, Murakami K, Nasu M. p53 expression in gastric mucosa with Helicobacter pylori infection. J Gastroenterol Hepatol 1998; 13: 215-219
30. Oda T, Murakami K, Nishizono A, Kodama M, Nasu M, Fujioka T. Long-term Helicobacter pylori infection in Japanese monkeys induces atrophic gastritis and accumulation of mutations in the p53 tumor suppressor gene. Helicobacter 2002; 7: 143-151
31. Sugiyama A, Maruta F, Ikteno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M. Helicobacter pylori infection enhances N-methyl-N-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res 1998; 58: 2067-2069
32. Tokieda M, Honda S, Fujioka T, Nasu M. Effect of Helicobacter pylori infection on the N-methyl-N-nitroso-N-nitrosoguanidine-induced gastric carcinogenesis in mongolian gerbils. Carcinogenesis 1999; 20: 1261-1266
33. Shimizu N, Inada K, Nakashima H, Tsukamoto T, Ikehara Y, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M. Helicobacter pylori infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. Carcinogenesis 1999; 20: 669-676
34. Hazell SL, Eichberg JW, Lee DR, Alpert L, Evans DG, Evans DJ, Graham DY. Selection of the chimpanzee over the baboon as a model for Helicobacter pylori infection. Gastroenterology 1992; 103: 848-854
35. Rauws EA, Langenberg W, Houthoff HJ, Zanen HC, Tytgat GN. Campylobacter pyloridis-associated chronic active antral gastritis. A prospective study of its prevalence and the effects of antibacterial and antulcer treatment. Gastroenterology 1988; 94: 33-40
36. Kimura K, Takemoto T. Endoscopic atrophy border. Endoscopy 1969; 1: 1-3
37. Lee A, Fox JG, Otto G, Murphy J. A small animal model of human Helicobacter pylori infection in nude mice. Gastroenterology 1990; 99: 1315-1323
38. Danon SJ, Moss ND, Larsson H, Arvidsson S, Ottosson S, Dixon MF, Lee A. Gastrin release and gastric acid secretion in the rat infected with either Helicobacter felis or Helicobacter heilmannii. J Gastroenterol Hepatol 1998; 13: 95-103
39. Marchetti M, Arico B, Burroni D, Figura N, Rappuoli R, Ghia P. Development of a mouse model of Helicobacter pylori infection that mimics human disease. Science 1995; 267: 1655-1658
40. Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of Helicobacter pylori infection: introducing the Sydney strain. Gastroenterology 1997; 112: 1386-1397
41. Cui G, Qvigstad G, Falkmer S, Sandvik AK, Kawase S, Waldum H. Spontaneous ECLomas in cotton rats (Sigmodon hispidus): tumours occurring in hypacidic/ hypergastrinaemic animals with normal parietal cells. Carcinogenesis 2000; 21: 23-27
42. Fox JG, Wishnok JS, Murphy JC, Tannenbaum SR, Correa P. MNNG-induced gastric carcinoma in ferrets infected with Helicobacter mustelae. Carcinogenesis 1993; 14: 1957-1961
43. Fox JG, Dangler CA, Sager W, Borkowski R, Gliatto JM. Helicobacter mustelae-associated gastric adenocarcinoma in ferrets (Mustela putorius furo). Vet Pathol 1997; 34: 225-229
44. Fox JG, Sheppard BJ, Dangler CA, Whary MT, Ihrig M, Wang TC. Germ-line p53-targeted disruption inhibits helicobacter-induced premalignant lesions and invasive gastric carcinoma through down-regulation of Th proinflammatory responses. Cancer Res 2002; 62: 696-702
45. Kim DH, Kim SW, Song YJ, Oh TY, Han SU, Kim YB, Joo HY, Cho YK, Kim DY, Cho SW, Kim MW, Kim JH, Hahn KB. Long-term evaluation of mice model infected with Helicobacter pylori: focus on gastric pathology including gastric cancer. Aliment Pharmacol Ther 2003; 18 Suppl 1: 1-24
46. Fox JG, Wang TC, Rogers AB, Pouthidhis T, Ge Z, Taylor N, Dangler CA, Israel DA, Krishna U, Gaus K, Peek RM Jr. Host and microbial constituents influence Helicobacter pylori-induced cancer in a murine model of hypergastrinemia. Gastroenterology 2003; 124: 1879-1889
47. Hirayama F, Takagi S, Kusugama H, Iwao E, Yokoyama Y, Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in mongolian gerbils infected with Helicobacter pylori. J Gastroenterol 1996; 31: 755-757
48. Honda S, Fujioka T, Tokieda T, Gotoh T, Nishizono A, Nasu M. Gastric ulcer, atrophic gastritis, and intestinal metaplasia caused by Helicobacter pylori infection in Mongolian gerbils. Sand J Gastroenterol 1998; 33: 454-60
49. Ikteno T, Ota H, Sugiyama A, Ishida K, Katsuyama T, Genta RM, Kawasaki S. Helicobacter pylori-induced chronic active gastritis.
gastritis, intestinal metaplasia, and gastric ulcer in Mongolian gerbils. Am J Pathol 1999; 154: 951-960

50 Suzuki H, Mori M, Seto K, Kai A, Kawaguchi C, Suzuki M, Suematsu M, Yoneta T, Miura S, Ishii H. Helicobacter pylori-associated gastric pro- and antioxidant formation in Mongolian gerbils. Free Radic Biol Med 1999; 26: 679-684

51 Tatematsu M, Yamamoto M, Shimizu N, Yoshikawa A, Fukami H, Kaminishi M, Oohara T, Sugiyama A, Ikeno T. Induction of glandular stomach cancers in Helicobacter pylori-sensitive Mongolian gerbils treated with N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine in drinking water. Jpn J Cancer Res 1998; 89: 97-104

52 Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. Gastroenterology 1998; 114: 1169-1179

53 Tanaka Y, Osugi H, Morimura K, Takekura M, Ueno M, Kaneko M, Fukushima S, Kinoshita H. Effect of duodenal gastric reflux on N-methyl-N'-nitro-N-nitrosoguanidine-induced glandular stomach tumorigenesis in Helicobacter pylori-infected Mongolian gerbils. Oncol Rep 2004; 11: 965-971

54 Abe H, Murakami K, Satoh S, Sato R, Kodama M, Arita T, Fujioka T. Influence of bile reflux and Helicobacter pylori infection on gastritis in the remnant gastric mucosa after distal gastrectomy. J Gastroenterol 2005; 40: 563-569

55 Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. Helicobacter pylori infection induces gastric cancer in mongolian gerbils. Gastroenterology 1998; 115: 642-648

56 Honda S, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M. Development of Helicobacter pylori induced gastric carcinoma in Mongolian gerbils. Cancer Res 1998; 58: 4255-4259

57 Hirayama F, Takagi S, Iwao E, Yokoyama Y, Haga K, Hanada S. Development of poorly differentiated adenocarcinoma and carcinoid due to long-term Helicobacter pylori colonization in Mongolian gerbils. J Gastroenterol 1999; 34: 450-454

58 Zheng Q, Chen XY, Shi Y, Xiao SD. Development of gastric adenocarcinoma in Mongolian gerbils after long-term infection with Helicobacter pylori. J Gastroenterol Hepatol 2004; 19: 1192-1198

59 Ogura K, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M. Virulence factors of Helicobacter pylori responsible for gastric diseases in Mongolian gerbil. J Exp Med 2000; 192: 1601-1610

60 Kagawa J, Honda S, Kodama M, Sato R, Murakami K, Fujioka T. Enterocarciomafin-like cell tumor induced by Helicobacter pylori infection in Mongolian gerbils. Helicobacter 2002; 7: 390-397

61 Shimizu N, Ikehara Y, Inada K, Nakanishi H, Tsukamoto T, Nozaki K, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M. Eradication diminishes enhancing effects of Helicobacter pylori infection on glandular stomach carcinogenesis in Mongolian gerbils. Cancer Res 2000; 60: 1512-1514

62 Hibi K, Mitomi H, Koizumi W, Tanabe S, Saigenji K, Okayasu I. Enhanced cellular proliferation and p53 accumulation in gastric mucosa chronically infected with Helicobacter pylori. Am J Clin Pathol 1997; 108: 26-34

63 Satoh K, Kihihi K, Kawata H, Tokumaru K, Kumakura Y, Ishino Y, Kawakami S, Inoue K, Kojima T, Satoh Y, Mutoh H, Sugano K. p53 expression in the gastric mucosa before and after eradication of Helicobacter pylori. Helicobacter 2001; 6: 31-36

64 Kodama M, Fujioka T, Murakami K, Okimoto T, Sato R, Watanabe K, Nasu M. Eradication of Helicobacter pylori reduced the immunohistochemical detection of p53 and MDM2 in gastric mucosa. J Gastroenterol Hepatol 2005; 20: 941-946

65 Murakami K, Fujioka T, Okimoto T, Mitsuishi Y, Oda T, Nishizono A, Nasu M. Analysis of p53 gene mutations in Helicobacter pylori-associated gastritis mucosa in endoscopic biopsy specimens. Scand J Gastroenterol 1999; 34: 474-477

66 Murakami K, Fujioka T, Kodama M, Honda S, Okimoto T, Oda T, Nishizono A, Sato R, Kubota T, Kagawa J, Nasu M. Analysis of p53 mutations and Helicobacter pylori infection in human and animal models. J Gastroenterol 2002; 37 Suppl 13: 1-5

67 Suzuki H, Miyazawa M, Nagashashi S, Mori M, Seto K, Kai A, Suzuki M, Miura S, Ishii H. Attenuated apoptosis in H pylori-colonized gastric mucosa of Mongolian gerbils in comparison with mice. Dig Dis Sci 2002; 47: 90-99

68 Crabtree JE, Court M, Aboshiki MA, Jeremy AH, Dixon MF, Robinson PA. Gastric mucosal cytokine and epithelial cell responses to Helicobacter pylori infection in Mongolian gerbils. J Pathol 2004; 202: 197-207

69 Suzuki H, Minegishi Y, Nomoto Y, Ota T, Masaoka T, van den Brink GR, Hibi T. Down-regulation of a morphogen (sonic hedgehog) gradient in the gastric epithelium of Helicobacter pylori-infected Mongolian gerbils. J Pathol 2005; 206: 186-197

70 van den Brink GR, Hardwick JC, Nielsen C, Xu C, ten Kate FJ, Glickman J, van Deventer SJ, Roberts DJ, Peppelenbosch MP. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. Gut 2002; 51: 628-633

71 Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. Science 2004; 306: 1568-1571