Short Communication

Naturally Occurring *Helicobacter pylori* Infection in the Cynomolgus Monkey (*Macaca fascicularis*) Used in Subclinical Studies

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Abstract: Recently, a high incidence of naturally occurring *Helicobacter pylori* (*H. pylori*)-related gastritis was reported in a cynomolgus monkey colony (Reindel *et al.*, 1999). We retrospectively examined 82 cynomolgus monkeys from three different countries. These monkeys were used in toxicological or pharmaceutical studies in our laboratory. Diffuse antral gastritis was observed in 49 of 58 (84%) animals of Group A in the Philippines, and two of 10 (20%) of Group C in Japan. In their antrum, the mucosal layer was thickened with lymphocytic and plasmacytic infiltration, and enlarged lymphoid follicles. However, no erosion or hemorrhage was seen in the lesions. *H. pylori*-like bacteria were detected by Warthin-Starry staining in almost all of the monkeys showing antral gastritis. No histological changes or *H. pylori*-like bacteria were detected in the animals of Group B in Indonesia. In conclusion, although the incidence of infection may be in part due to the breeder, natural infection of *H. pylori* was confirmed in cynomolgus monkeys. Thus, this species as well as rhesus monkeys may be useful in the study of *H. pylori* infection. Moreover, because of its high incidence, the colony in the Philippines may be suitable for an animal model, as it is comparable with natural infection in humans. (J Toxicol Pathol 2001; 14: 45–49)

Key words: *Helicobacter pylori* infection, cynomolgus monkeys, antral gastritis

Helicobacter pylori (*H. pylori*) is a serious pathogen in humans causing chronic atrophic gastritis and persistent ulcer, and is related to some gastric tumors¹. The same gastric changes as those in humans have been reported in non-human primates by natural^{2–7} or experimental^{2, 8–13} infection with this pathogen. There have been many studies of *H. pylori* infection in rhesus monkeys (*Macaca mulatta*)^{2–7, 12, 13}, and this monkey species is regarded as a good human model for infection with this pathogen. Despite belonging to the same genus, *Macacus*, cynomolgus monkeys (*Macaca fascicularis*) are less susceptible to *H. pylori* infection than rhesus monkeys². Therefore, there have been few reports concerning infection of cynomolgus monkeys with this pathogen^{2, 10}.

Recently, a high incidence of naturally occurring *H. pylori*-related gastritis was reported in a cynomolgus monkey colony of a pharmaceutical research laboratory¹⁴. According to the article, the monkeys originated from the

Philippines. We retrospectively examined formalin-fixed stomach specimens of 82 cynomolgus monkeys, which were used and sacrificed in toxicological or pharmaceutical studies from 1995 to 1998 (Table 1) in our laboratory. The test substances had no effects on the stomach. The monkeys were obtained from commercial breeders in three different countries; 58 animals (Group A) from the Philippines (Simian Conservation Breeding & Research Center, Inc.), 14 animals (Group B) from Indonesia (C. V. Primates, Inc.), and 10 animals (Group C) from Japan (Japan E. D. M., Inc.). Group A in the Philippines and Group C in Japan adopted a colony-breeding system, and the monkeys of Group A may have come from the same colony as that of a previous study¹⁴. The monkeys of Group B were captured and raised in Indonesia.

Two specimens of the gastric fundus and antrum from each animal were stained with hematoxylin and eosin (HE), and Warthin-Starry stain. Selected specimens were immunohistochemically stained with a polyclonal rabbit anti-*H. pylori* antibody (Signet, Dedham, MA, USA) using the labeled streptavidin-biotin method (Dako LSAB Kit; Dako Co., Carpinteria, CA, USA). Selected samples from the gastric mucosa of the fundus and antrum were taken from the formalin-fixed tissue for transmission electron

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Table 1. Histopathological Findings of the Stomach in Cynomolgus Monkeys

	Group A	Group B	Group C
Number of monkeys examined (male, female)	58 (30, 28)	14 (7, 7)	10 (-, 10)
Mean age (range)	3.8 (3-5)	7.6 (5-13)	5.2 (5-6)
	M&F (M, F)#	M&F (M, F)	M&F (M, F)
Diffuse antral gastritis	49 (25, 24)	0	2 (-, 2)
Lymphoplasmacytic infiltration	50 (26, 24)	0	2 (-, 2)
Mucosal hyperplasia	46 (22, 24)	0	2 (-, 2)
Enlarged lymph follicle	43 (22, 21)	1 (1, 0)	1 (-, 1)
Microabscess	23 (9, 14)	0	0
Fundic gastritis	12 (6, 6)	0	0
Bacteria detected by Warthin-Starry staining			
<i>H. pylori</i> -like in the antrum	51 (27, 24)	0	2 (-, 2)
<i>H. heilmannii</i> -like in the fundus	54 (27, 27)	14 (7, 7)	10 (-, 10)

#M&F (M, F), Total number of males and females (Males, Females).

Fig. 1 Antral mucosa of cynomolgus monkeys. a: No particular change is observed in a monkey of Group B. b: Thickening of the mucosa due to marked lympho-plasmacytic infiltration. HE staining, $\times 100$.

microscopy.

No macroscopic evidence of disease was observed in any of the 82 monkeys. Diffuse antral gastritis was observed in 49 of 58 (84%) animals regardless of gender, and two of 10 (20%) animals in Groups A and C, respectively. In the antrum of these animals, numerous lymphocytes and plasma cells, some of which changed to Mott cells, were infiltrated into the lamina propria and into the epithelial layer of the mucosa (Figs. 1b and 2). Microabscesses were occasionally observed within the upper antral pits and glands. However, no mucosal erosion or hemorrhage was seen. Enlarged lymphoid follicles with a prominent germinal center were formed throughout the lamina propria and in the submucosal

layer. Although the mucosa was thickened and its surface, pits, and glandular epithelium were hyperplastic. Furthermore, the epithelial cell height was diminished due to a decrease in the mucus content. Some bacterial clumps were visible within the upper pits and glands (Fig. 2), but the disseminated organisms could not be readily detected by HE staining. In 12 of 58 monkeys of Group A, such gastritis extended to the fundic area. However, other monkeys in both the other groups showed less marked alterations in the fundus. No changes were detected in Group B. Warthin-Starry staining revealed two morphologically distinct bacteria in the antral (Fig. 3a) and fundic (Fig. 4a) mucosa. Moreover, both organisms were immunohistochemically

Fig. 2 Higher magnification of Fig. 1b. A bacterial clump can be seen within the upper pit (arrows). HE staining, $\times 400$.

positive with the anti-*H. pylori* antibody (Figs. 3b, and 4b). Concurrent infection with *H. pylori* and *H. heilmannii*-like organisms has been reported^{5, 6, 14}. *H. heilmannii* can not be cultured *in vitro* and the organism can only be detected by morphological examination¹⁴. Although its pathogenicity has been recently reported in humans¹⁵⁻¹⁷, it has not been clear in non-human primates^{5, 6, 14}. In this study, these two bacteria could be distinguished from each other based on their morphology and location, as previously reported in cynomolgus¹⁴ and rhesus monkeys^{5, 6}. *H. pylori*-like bacteria, which were short in length, curved, and rod-shaped, were located in the gastric pits and the upper gastric glands of the antral mucosa. The monkeys, which were positive for *H. pylori*-like bacteria on Warthin-Starry staining, mostly corresponded with those showing antral gastritis. *H. heilmannii*-like bacteria, which were long, tightly coiled and spiral in shape, were found within the lumen of the pits, glands, and surface mucus of the fundic mucosa. The organisms were especially prevalent in the cytoplasm of parietal cells. These organisms were detected in almost all monkeys in this study. However, no pathological changes were observed and the prevalence of these organisms was deemed to have no relation to the antral gastritis. Ultrastructural examination also showed two different spiral bacteria. *H. pylori*-like bacteria with no coiling, measuring approximately $3\ \mu\text{m} \times 1.3\ \mu\text{m}$, were found in the lumen of the antral pits (Fig. 5a). *H. heilmannii*-like bacteria were present in the fundic glands and in the parietal cells, and had four to eight spirals measuring $8\ \mu\text{m} \times 1\ \mu\text{m}$ at maximum length (Fig. 5b). Many flagellae were seen around both organisms, but in this study, we could not confirm their polarity as previously reported^{1, 4}.

Fig. 3 *Helicobacter pylori*-like organisms (arrows) on the gastric gland surface. a: Warthin-Starry staining. b: Anti-*Helicobacter pylori* antibody immunostaining counterstained with methyl green. $\times 600$.

In addition, five cynomolgus monkeys underwent gastroduodenal endoscopic examination. They were simultaneously obtained from the same colony as Group A and maintained without any treatment for two years in our laboratory. The superficial mucus and mucosal samples taken from the pyloric antrum with endoscopy were smeared onto modified Belo-Horizonte medium (M-BHM; Nikken Bio Medical Laboratory Co., Ltd., Kyoto, Japan) and cultured for five days at 35°C in a microaerophilic

Fig. 4 *Helicobacter heilmannii*-like organisms (arrows) within the lumen and in the parietal cells of the fundic glands. a: Warthin-Starry staining. b: Anti-*Helicobacter pylori* antibody immunostaining counterstained with methyl green. $\times 600$.

atmosphere. *H. pylori*-like organisms were detected in three of the five monkeys, and the organisms in these animals were positive for urease, catalase, and oxidase activities. Furthermore, the isolated organisms were sensitive to both amoxicillin (minimum inhibitory concentration (MIC) = 0.025–0.05 $\mu\text{g/mL}$) and clarithromycin (MIC = 0.025 $\mu\text{g/mL}$). These microbiological findings were consistent with the results in previous reports^{2, 10}. So it was strongly indicated that the organisms were *H. pylori*.

Fig. 5 Electron microscopy. a: *Helicobacter pylori*-like organisms with no coil. b: *Helicobacter heilmannii*-like organisms with tight spirals. Many flagellae are seen around both organisms (arrows). Bar = 1 μm .

The results of this study indicated that the cynomolgus monkeys of Group A were highly infected with *H. pylori*. Group A was from the Philippines, and possibly the same colony as that in a previous study¹⁴. If this was the case, we may be able to reproduce and confirm their results. In addition, this study demonstrated natural infection with *H. pylori* in another country. It is, therefore, unlikely that cynomolgus monkeys are less susceptible to *H. pylori* infection than rhesus monkeys. The apparent higher susceptibility of rhesus monkeys may simply be because they are more commonly used in biomedical research than cynomolgus monkeys¹⁴.

H. pylori infection is common in captive rhesus monkeys and natural transmission is by fecal-oral and oral-oral routes, and by close contact during breast feeding¹³. Such transmission can readily occur in colony-bred

monkeys, even cynomolgus monkeys. Furthermore, as human-to-human transmission of the organism via endoscopy has been reported¹⁸, inadequate sterilization of gastric gavage tubes is likely to be a significant route of transmission in oral administration studies. In addition, this and the previous¹⁴ studies revealed that the colony in the Philippines had a higher incidence of *H. pylori* infection. The natural infection in the colony might be similar to the epidemiological prevalence in humans. So clinical and laboratory surveys of the monkeys in their life span are important to study *H. pylori*-infectious diseases.

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