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Gastric Mucosal Interleukin-17 and -18 mRNA Expression in *Helicobacter pylori*-Induced Mongolian Gerbils

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Abstract

Helicobacter pylori infection causes characteristic mucosal infiltration of inflammatory cells, resulting in the development of peptic ulcers and gastric cancer in approximately 10% of cases. Different clinical expressions of the infection may reflect different patterns of cytokine expression. Interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , IL-17, and IL-18 have been reported to be involved in *H. pylori*-induced gastric mucosal inflammation, but the details and relation to different patterns of inflammation remain unclear. Moreover, the proinflammatory virulence factor outer inflammatory protein (OipA) was reported to be associated with gastric mucosal inflammatory cytokine levels. To clarify these findings, Mongolian gerbils were infected for up to 12 months with wild-type *H. pylori* 7.13 or with isogenic *oipA* mutants for 3 months, and mucosal cytokines (IL-1 β , IL-17, IL-18, and TNF- α) mRNA levels were then assessed using real-time RT-PCR. Antral mucosal IL-1 β and IL-18 mRNA levels peaked 1 month after infection, whereas the peak of TNF- α mRNA was at 6–12 months; IL-17 levels peaked at 12 months. The inflammatory cell infiltration and mRNA levels of all cytokines studied were significantly lower in *oipA* mutants than in wild-type-infected gerbils. Mucosal IL-1 β , IL-17, and TNF- α expression, but not that of IL-18, were significantly associated with the grade of inflammatory cell infiltration. The pattern of increased inflammatory cytokines differed relative to the phase of the infection and pattern of inflammation. OipA appears to play a role in IL-1 β , IL-17, and TNF- α expression and the resulting inflammation.

Helicobacter pylori infection is characterized pathologically by a marked infiltration of neutrophils, lymphocytes, monocytes, and plasma cells into the gastric mucosa. Migration and activation of these inflammatory cells into the gastric mucosa results in production of a number of pro-inflammatory cytokines, including IL-1 β , IL-6, IL-8, IL-17, IL-18, TNF- α , and IFN- γ .^(1–4) The risk of a clinical outcome such as gastric cancer or peptic ulcer disease is thought to result from interactions between the bacteria, the environment, and host genetic factors. For example, polymorphisms of pro-inflammatory cytokine genes such as *IL-1B*, *IL-8*, *IL-10*, and *TNF-A* resulting in an enhanced inflammatory response of gastric mucosa have been associated with an increased risk of gastric cancer and peptic ulcer.^(5–10)

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Mongolian gerbils infected with *H. pylori* have proven to be an excellent model for studying *H. pylori* disease pathogenesis. As in humans, the infection initially presents as an antral-pre-dominant gastritis, followed by advancement of the inflammation into the corpus gastritis, eventually leading to atrophic gastritis with metaplasia, gastric ulcers,^(11–15) and rarely even gastric cancer.^(16,17) Maximum levels of mucosal IL-1 β and IFN- γ mRNA occur within 4 weeks after infection. In contrast, IL-4, IL-6, and IL-10 levels peak between 8 and 26 weeks.⁽⁴⁾ IL-17 and IL-18 have recently been associated with *H. pylori*-related gastric mucosal inflammation and gastroduodenal disease risks, but the time course and relationship with bacterial virulence factors remain unknown.^(18,19)

The risks of *H. pylori*-induced gastric inflammation, atrophy, metaplasia, and malignancy have been related to the presence of *H. pylori*-associated virulence factors.^(20–22) OipA is a proinflammatory response-inducing protein⁽²⁰⁾ associated with high *H. pylori* density and more severe neutrophil infiltration.⁽²¹⁾ In recent work, *oipA*-negative strains were reported to have a decreased ability to induce gastric inflammation and disease in relation to decreasing *H. pylori* density in Mongolian gerbils.⁽²³⁾ Therefore, OipA mediates adherence of *H. pylori* to gastric epithelial cells and contributes to the pathogenesis of gastroduodenal diseases.^(20–22)

The present study investigated several inflammatory cytokine profiles in the acute and chronic phases of *H. pylori* infection of Mongolian gerbils using real-time RT-PCR, and examined the role of OipA in *H. pylori*-induced gastric mucosal inflammatory cytokine production in the Mongolian gerbil.

Materials and Methods

Animals

Six-week-old male specific pathogen-free Mongolian gerbils (MGS/Sea) with average weight of approximately 50 g were purchased from Charles River Laboratories (Wilmington, MA, USA). They were housed in polypropylene cages on hard wood chip bedding in groups of five per cage, and caged under a 12 : 12 h L : D cycle. The animals used in this study were cared for in accordance with our institutional guidelines. The experimental protocol was approved by the Animal Care Committee of Baylor College of Medicine and Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX, USA.

Bacteria and Bacterial Inoculation

We used *H. pylori* strain 7.13, which was provided by Dr R. M. Peek and has been shown to cause reproducible mucosal damage.⁽²³⁾ We also used its isogenic *oipA* mutants, which were constructed as previously described.⁽²⁰⁾

H. pylori were grown in brain heart infusion broth (BD, Sparks, MD, USA) containing 15% FBS for 20 h at 37°C under microaerobic conditions (12% CO₂) and saturated humidity (95%), shaking gently. After fasting, each animal was inoculated once daily for 3 days with 1 mL medium of *H. pylori* (10⁹CFU/mL) using a metal stomach catheter. The control group was inoculated three times with brain heart infusion broth medium.

Experimental Design

We carried out two experiments. Experiment 1 studied animals during the first 12 months of infection. Mongolian gerbils were randomly divided into two groups: group A was uninfected (negative control) and group B was infected with *H. pylori* 7.13. For each time point, five to seven gerbils were killed at 1, 3, 6, and 12 months after inoculation of *H. pylori* 7.13 strain (group B). In addition, three age- and sex-matched uninfected gerbils were

killed at each time point. Experiment 2 lasted 3 months (group A). Mongolian gerbils were inoculated with *H. pylori* 7.13 *oipA* mutants, as group C, and were killed after 1 or 3 months.

At necropsy, the stomach was opened along the greater curvature; the longitudinal half of the stomach was fixed in 10% buffered formalin for histological examination. The other half was divided further into the pyloric gland mucosa (antrum) and the fundic (corpus) and stored at -80°C . In addition, small amounts of gastric mucosa were taken for culture of *H. pylori*.⁽²⁴⁾ DNA was extracted from the mucosa if *H. pylori* were not detected by culture, and PCR was carried out for the *oipA* gene.⁽²⁴⁾

Histological Examination

The stomach was fixed in 10% buffered formalin and cut along the longitudinal axis. The stomach was processed by standard methods and embedded in paraffin following standard procedures. The tissue was sectioned at 4 μm for HE and Genta staining. The degree of inflammation assessed by the number of mononuclear cell (MNC) and polymorphonuclear cell (PMN) infiltration and *H. pylori* density were graded from 0 (absent/normal) to five (maximal intensity).⁽²⁵⁾ Each strip of gastric mucosa was scored and the results were averaged.

Sequence Analysis of Mongolian Gerbil IL-17

To identify the Mongolian gerbil *IL-17* mRNA sequence, we selected conservative parts of the sequence among mouse, rat, and human *IL-17* mRNA.⁽²⁶⁻²⁸⁾ We chose a forward primer (IL-17-2F, 5'-CCA GAA GGC CCT CAG ACT AC-3') and a reverse primer (IL-17-R, 5'-AGG ACC AGG ATC TCT TGC TG-3') for PCR, and amplified a segment of approximately 236 bp from the cDNA. PCR fragments were purified and directly sequenced at Macro-gen (Seoul, Korea).

Real-Time PCR Analysis

After the gastric mucosal tissues divided into the antrum and the corpus was homogenized, total RNA was isolated using RNeasy Mini Kits (Qiagen, Valencia, CA, USA) and 0.5 μg RNA from each sample was reverse-transcribed using 50 U SuperScript III RT (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' protocols. One μL of cDNA was amplified by PCR using the qPCR MasterMix Plus for SYBR Green I Kit (Eurogentec, San Diego, CA, USA) and specific primers for each cytokine and β -actin (Table 1). Each PCR cycle consisted of a denaturation step (94°C , 1 min), an annealing step (55°C , 30 s), and an elongation step (72°C , 30 s). There were a total of 50 cycles, followed by an additional extension step (72°C , 7 min).

Data Analysis

Data are presented as medians and range or as mean and SE depending on whether the distribution was normal. Statistical tests included Mann–Whitney analysis or Student's *t*-test, depending on the data set of concern. A *P*-value of <0.05 was accepted as statistically significant. Calculations were carried out using StatView 5.0 statistical software (SAS Institute, Cary, NC, USA).

Results

Effect of *H. Pylori* infection on Macroscopic and Pathological Findings

All animals treated with wild-type *H. pylori* 7.13, or its *oipA* mutant, were infected as documented by culture and/or histology. Following inoculation of the wild-type 7.13 strain,

gastric ulcers were observed in four of nine gerbils (44%) at 3 months, three of 10 gerbils (30%) at 6 months, three of five gerbils (60%) at 12 months in the antrum, and were observed in five of nine gerbils (56%) at 3 months, and one of 10 gerbils (10%) at 6 months in the corpus (Table 2). Most gerbils developed intestinal metaplasia, which had higher potential to develop into gastric cancer, at 6 and 12 months. No ulcers and intestinal metaplasia were seen in Mongolian gerbils infected with the 7.13 *oipA* mutants (Table 2).

Mucosal infiltration with MNC and PNC was rare to absent in control animals. At 3 and 6 months after inoculation of the wild-type 7.13 strain, there was marked infiltration of both MNC and PNC observed mainly in the submucosa, reaching maximum levels in the antrum (Fig. 1A,B). In the corpus, the infiltration of MNC and PNC reached maximum levels at 6 and 12 months (Fig. 2A,B). Focal aggregates of lymphocytes at the glandular border of the antrum and the corpus were observed in Mongolian gerbils infected with the 7.13 wild-type strain. The *H. pylori* density and thickness of the gastric antrum wall in the wild-type-inoculated group were significantly greater than in controls during the 12-month experimental period (Fig. 1C,D). The *H. pylori* density did not increase significantly in the corpus (Fig. 2C), although the inflammation score and thickness of the gastric wall in the corporal mucosa were enhanced after inoculation. Inflammation remained predominantly antral (Figs 1,2); however, the area of the corpus decreased significantly in parallel with an expansion of the pyloric mucosa.

We investigated the role of OipA in terms of gastric inflammation following inoculation of *oipA* mutants over a 3-month period (experiment 2). No gastric ulcers or erosions were seen in *oipA* mutant-infected gerbils during the 3-month infection. Moreover, *oipA* mutants induced a mild inflammation compared to wild type. When compared with non-infected animals, the PMN scores in the antrum were significantly higher in *oipA* mutants than controls at 3 months after inoculation (Fig. 1).

Sequence Analysis of the Mongolian Gerbil *IL-17* Gene

Partial gerbil-specific *IL-17* cDNA sequences were successfully cloned. The sequences of Mongolian gerbil and murine *IL-17* were closely related (157 of 185 [85.8%] at the nucleotide level). A high degree of homology was also observed for *IL-17* sequences among the rat and Mongolian gerbil (151 of 183 [82.5%] at the nucleotide level) (Fig. S1).

Gastric mucosal cytokine mRNA levels in Mongolian gerbils by *H. pylori* infection

IL-1 β , IL-17, IL-18, and TNF- α mRNA levels were not induced in the gastric mucosa of uninfected animals throughout the 12-month observation period. IL-17 levels remained similar in the corporal and antral mucosa throughout the observation period, and gradually increased over the time course in both the antrum and the corpus (Fig. 3A). IL-1 β and IL-18 transcripts were strongly induced in the antrum 1 month after *H. pylori* 7.13 wild-type inoculation and returned to the baseline by 6 months (Fig. 3B,C). The IL-1 β and IL-18 mRNA levels in the corpus in wild-type infection were lower than those in the antrum, reaching maximal levels 1–6 months after inoculation (i.e. at a later time than in the antrum) (Fig. 3B,C). The TNF- α mRNA levels in the corpus were predominantly higher than those in antral mucosa, with a maximum at 6 months (Fig. 3D).

When infected with *oipA* knockouts, the IL-1 β , IL-18, and TNF- α mRNA levels were significantly lower than those in wild type from 1 to 3 months, in both the antrum and the corpus (Figs 4,5). However, IL-17 mRNA levels were similar to those with wild-type infection during the entire observation period in both the antrum and the corpus (Figs 4A, 5A).

In infected gerbils, IL-1 β mRNA levels correlated with TNF- α mRNA levels in the corpus ($r = 0.41$) ($P = 0.04$) and antral ($r = 0.73$) ($P < 0.01$) mucosa (Table 3). There were also significant correlations between IL-17 mRNA levels and IL-1 β ($r = 0.43$, $P = 0.02$) or TNF- α ($r = 0.64$, $P < 0.01$) mRNA in the corporal mucosa (Table 3). However, there was no correlation between IL-18 mRNA levels and mRNA levels of other cytokines in either the antral or corporal mucosa.

Cytokine mRNA and inflammatory cell infiltration in gastric mucosa of gerbils infected with the 7.31 wild-type *H. pylori* strain

In the corpus, both PNC and MNC scores correlated with IL-17 mRNA levels ($r = 0.481$ and 0.514 , respectively) (Fig. 6). These scores also correlated with IL-1 β and TNF- α mRNA levels in the corpus (Fig. 6). In the antral mucosa, although both PNC and MNC scores correlated with three different cytokine mRNA levels, the correlations were weaker than in the corpus (data not shown). In contrast, these scores were independent of IL-18 mRNA levels in the corpus and the antrum (data not shown).

Expression of cytokine mRNA in gastric ulcer patients

IL-1 β , IL-17, and TNF- α mRNA levels were significantly greater in the ulcer group than in the non-ulcer group ($P < 0.01$ for each) (Fig. 7). However, there was no relationship between the IL-18 mRNA levels and clinical presentation.

Discussion

The gastric mucosa responds to *H. pylori* infection by producing pro-inflammatory cytokines such as IL-1 β , IFN- γ , IL-6, IL-8, TNF- α , and macrophage inflammatory protein-1 (MIP-1), which are all involved in orchestrating the cellular immune response.^(1–4,15) Animal models are ideal to study the time course and details of this response. One problem with the gerbil model has been the lack of reagents. This problem has been partially overcome by partial cloning of the relevant cytokines. We cloned partial sequences of Mongolian gerbil IL-17 mRNA for measuring the IL-17 mRNA levels using real-time RT-PCR. To our knowledge this is the first use of real-time RT-PCR for the detection of IL-17 and IL-18 mRNA in *H. pylori*-infected Mongolian gerbils.

Mongolian gerbils infected with *H. pylori* develop an antral-predominant gastritis, which over time progresses to involve the corpus.^(11–15) Maximal gastric antral mucosal inflammation in atrophy appeared early in the course of chronic *H. pylori* infection and then decreased dramatically.⁽⁴⁾ The acute inflammation was paralleled by mucosal cytokine expression, especially of IL-1 β . In contrast, gastric corporal mucosal inflammation plateaued and remained elevated for long periods after reaching maximum levels at approximately 3–6 months.⁽⁴⁾

We previously showed that chronic gastric inflammation tended to parallel IFN- γ expression.⁽⁴⁾ This study extended those findings by showing a relationship with both TNF- α and IL-17. Chronic atrophic gastritis is thought to be the precursor to gastric ulcer and gastric cancer such that the risk of developing gastric cancer parallels the extent and severity of gastric mucosal atrophy.^(29–33) Polymorphisms in these pro-inflammatory genes (i.e. *TNF-A*-1031 T/C, -863 C/A, and -857 C/T and IL-17F 7488 T/C) result in high transcriptional promoter activity, and increased inflammation is associated with an increased risk of *H. pylori*-associated gastric cancer and peptic ulcer development.^(6,10,34,35)

IL-17 produced from the T memory compartment is a member of an emerging family of inflammatory cytokines whose biological activities remain incompletely defined.⁽³⁶⁾ IL-17 has pleiotrophic activities, including the induction of inflammatory cytokines (e.g. TNF- α

and IL-1 β), and plays a key role in the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis and osteoarthritis synovial.⁽³⁷⁾ Moreover, IL-17 stimulates IL-8 release in gastric epithelial cells and facilitates the chemotaxis of neutrophils.^(38,39) Clinically, IL-17 levels have been shown to be increased in the gastric mucosa of *H. pylori*-infected patients.^(38,40) Recently, Shiomi *et al.* reported that the PMN infiltration level in IL-17^{-/-} mice infected with *H. pylori* was significantly lower than that in wild-type mice.⁽⁴¹⁾ In the present study, we confirmed that gastric mucosal IL-17 levels in both the antrum and body were increased in Mongolian gerbil after *H. pylori* infection, especially in the chronic phase of *H. pylori* infection. Moreover, gastric IL-17 mRNA levels correlated with the inflammatory cell infiltration level as well as gastric ulcer risk. These results suggest that IL-17 plays an important role in the inflammatory response to *H. pylori* infection and ultimately influences the outcome of *H. pylori*-associated disease.

IL-18 is a T-helper lymphocyte type 1 (Th1) cytokine in the IL-1 superfamily and an IFN- γ -inducing factor.⁽⁴²⁾ IL-18 mRNA levels have been shown to be increased in human *H. pylori* infection.^(18,43) IL-18 levels have also been reported to be a postoperative prognostic determinant in patients with gastric cancer.⁽⁴⁴⁾ *In vitro* studies have shown that IL-18 mRNA levels are markedly increased in *H. pylori*-infected epithelial cells and monocytes.⁽¹⁹⁾ Some studies in humans have reported that IL-18 protein and/or mRNA levels correlate with the severity of gastric inflammation;^(18,19) however, other studies have reported that IL-18 mRNA levels are independent of *H. pylori* infection.⁽⁴⁵⁾ Gastric mucosal IL-18 levels increased in Mongolian gerbil after *H. pylori* infection; however, IL-18 mRNA levels did not correlate with the mRNA levels of IL-1 β , IL-17, or TNF- α , or with the degree of inflammatory cell infiltration. There was also no significant difference in IL-18 mRNA levels between gerbils with gastric ulcer or gastritis. However, IL-18 mRNA levels increased after wild-type infection but not after *oipA* mutant infection. Understanding the role of IL-18 in human or gerbil *H. pylori* infection will require additional study.

oipA 'on' strains attach more tightly to the gastric mucosa and lead to the effects of enhancing gastric inflammation than do *oipA* 'off' strains.^(21,24,46) *H. pylori* virulent factors, such as *cagA* status and *vacA* s1, m1, and i1 genotypes, are significantly associated with increased scores for gastric mucosal atrophy and neutrophil infiltration relative to patients with lower virulence genotypes. We confirmed the observation that *oipA* mutants did not induce gastric inflammation in Mongolian gerbils. However, recently Franco *et al.* reported that there were no differences in the severity of inflammation between Mongolian gerbils infected with 7.13 wild type and its *oipA* mutant, and that all gerbils developed moderate-to-marked gastritis with frequent lymphoid follicle formation and ulcer, but *oipA*-negative strains had preventive effects for gastric dysplasia and cancer.⁽²³⁾ These observations suggest that because gastric mucosal inflammatory cytokine levels are important factors in the development of diseases, Mongolian gerbils infected with the *oipA* mutant did not enhance inflammatory cytokines, irrespective of the inflammatory cell infiltration level. IL-1 β , IL-17, IL-18, and TNF- α mRNA levels in *oipA* mutant-infected Mongolian gerbils were also significantly lower than in gerbils infected with wild-type strains; the status of *oipA* determined the gastric inflammation-related inflammatory cytokine levels, resulting in gastric ulcer and gastric cancer development.^(21,24,46)

In conclusion, we demonstrated that IL-1 β plays important roles in the acute phase of gastric inflammation after *H. pylori* infection and that TNF- α and as well as IL-17 is important in the maintenance and regulation of chronic gastric inflammation in *H. pylori*-infected Mongolian gerbils. Therefore, cytokine mRNA levels differed at each phase of gastric inflammation, and might be related to the development of specific clinical outcomes. Moreover, analysis of the inflammatory cytokine levels in gastric mucosa around the ulcer,

metaplasia, or cancer is useful for evaluating the role of cytokines in disease development in *H. pylori*-infected Mongolian gerbils; however, further study will be required.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IFN	interferon
IL	interleukin
MNC	mononuclear cell
OipA	outer inflammatory protein
PMN	polymorphonuclear cell
TNF	tumor necrosis factor

Fig. 1. (A,B) The inflammatory cell infiltration, (C) *Helicobacter pylori* density, and (D) gastric wall thickness in the gastric antral mucosa of Mongolian gerbils infected with *H. pylori* 7.13 wild type and outer inflammatory protein (*oipA*) isogenic mutants (KO). In *H. pylori* 7.13 wild-type infection, the mononuclear cell (MNC) and polymorphonuclear cell (PMN) level, *H. pylori* density, and gastric wall thickness significantly increased over time. * $P < 0.05$ compared with month 0.

Fig. 2.

(A,B) The inflammatory cell infiltration, (C) *Helicobacter pylori* density, and (D) gastric wall thickness in the gastric corporal mucosa of Mongolian gerbils infected with *H. pylori* 7.13 wild type and outer inflammatory protein (*oipA*) isogenic mutants (KO). In *H. pylori* 7.13 wild-type infection, the mononuclear cell (MNC) and polymorphonuclear cell (PMN) level and gastric wall thickness significantly increased over time. * $P < 0.05$ compared with month 0.

Fig. 3. Gastric antral and corporal mucosal cytokine mRNA levels in Mongolian gerbils infected with *Helicobacter pylori* 7.13 wild-type strain for 12 months. The maximum interleukin (IL)-1 β and IL-18 mRNA levels occurred 1 month after *H. pylori* 7.13 wild type inoculation. The levels of tumor necrosis factor (TNF)- α and IL-17 occurred 2 and 12 months after, respectively, and therefore the natural history of each cytokine differed. * $P < 0.05$ compared with month 0, and # $P < 0.05$ compared with corpus.

Fig. 4. Cytokine mRNA levels in the Mongolian gerbil gastric antral mucosa infected with *Helicobacter pylori* 7.13 wild type or its outer inflammatory protein (*oipA*) isogenic mutants (KO) for 3 months. Cytokine mRNA levels in 7.13 wild type were higher throughout the experimental period. * $P < 0.05$ compared with month 0. IL, interleukin; TNF, tumor necrosis factor.

Fig. 5. Cytokine mRNA levels in the Mongolian gerbil gastric corporal mucosa infected with *Helicobacter pylori* 7.13 wild type or its outer inflammatory protein (*oipA*) isogenic mutants (KO) for 3 months. Cytokine mRNA levels in 7.13 wild type were higher throughout the experimental period. * $P < 0.05$; compared with month 0. IL, interleukin; TNF, tumor necrosis factor.

Fig. 6.

The correlations between cytokine mRNA levels and inflammatory cell infiltration scores (x-axes) in corporal mucosa in Mongolian gerbils infected with the *Helicobacter pylori* 7.13 wild-type strain. Interleukin (IL)-1 β , IL-17, and tumor necrosis factor (TNF)- α mRNA levels were significantly correlated with inflammatory cell infiltration scores (r -values 0.409–0.590). MNC, mononuclear cell; PMN, polymorphonuclear cell.

Fig. 7.

Gastric mucosal cytokine levels in Mongolian gerbils in the ulcer and non-ulcer groups. Interleukin (IL)-1 β , IL-17, and tumor necrosis factor (TNF)- α mRNA levels in the ulcer group were higher than in the non-ulcer group. * $P < 0.05$ versus the non-ulcer group.

Table 1
Primer pairs used in this study

Cytokine	Sequence (5' to 3')
IL-1 β	
Forward	GGC AGG TGG TAT CGA TVA TC
Reverse	CAC CTT GGA TTT GAC TTC TA
IL-17	
Forward	AGC TCC AGA GGC CCT CGG AC
Reverse	AGG ACC AGG ATC TCT TGC TG
IL-18	
Forward	GCT GGC TGT AAC CCT CTC TG
Reverse	TTC CTC CTT TTG GCA AGC TA
TNF- α	
Forward	GCT CCC CCA GAA GTC GGC G
Reverse	CTT GGT GGT TGG GTA CGA CA
β -Actin	
Forward	TCC TCC CTG GAG AAG AGC TA
Reverse	CCA GAC AGC ACT GTG TTG GC

IL., interleukin; TNF, tumor necrosis factor.

Table 2
Incidence of gastric ulcer after inoculation *Helicobacter pylori*

Strain	Period (months)	Number	Peptic ulcer Corpus	Antrum
Wild type	1	6	0 (0%)	0 (0%)
Wild type	3	9	5 (55%)	4 (44%)
Wild type	6	10	1 (10%)	3 (30%)
Wild type	12	5	0 (0%)	3 (60%)
<i>oipA</i> mutant	1	6	0 (0%)	0 (0%)
<i>oipA</i> mutant	3	5	0 (0%)	0 (0%)

Table 3
Association among different cytokines in the gastric mucosa

	Cytokine			
	IL-1 β	IL-17	IL-18	TNF- α
Corpus				
IL-1 β	1.00	0.43 ($P = 0.02$)	NP	0.41 ($P = 0.04$)
IL-17	—	1.00	NP	0.64 ($P < 0.01$)
IL-18	—	—	1.00	NP
TNF- α	—	—	—	1.00
Antrum				
IL-1 β	1.00	NP	NP	0.73 ($P < 0.01$)
IL-17	—	1.00	NP	NP
IL-18	—	—	1.00	NP
TNF- α	—	—	—	1.00

The data showed Pearson's correlation value (P -value). IL, interleukin; NP, no significant correlation; TNF, tumor necrosis factor.