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Relationship between gastric mucosal IL-8 levels and histological gastritis in patients with *Helicobacter pylori* infection

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To determine the role of host immune responses in *H. pylori* infection, we examined the relationship between gastric mucosal IL-8 levels and histological gastritis in patients with *H. pylori* infection. Biopsy tissue obtained from 99 patients were homogenized and mucosal IL-8 levels measured by ELISA. The gastric mucosal IL-8 levels in both the antrum and corpus were higher in patients with *H. pylori* than in *H. pylori* negative patients. IL-8 levels in the corpus but not the antrum correlated with the severity of the atrophy. The *IL-1B* polymorphism had no influence on the degree of IL-8 production. These findings indicate that IL-8 production is independent of *IL-1B* polymorphisms and IL-8 may play an important role in the development of atrophic gastritis.

Key words: *H. pylori* infection, interleukin-8, Updated Sydney System

INTRODUCTION

Helicobacter pylori is a spiral bacterium that colonizes the human stomach [1]. *H. pylori* plays a critical role in the pathogenesis of peptic ulcers and the chronic inflammatory process, which may be linked to gastric cancer and gastric lymphoma [2, 3]. However, why patients with *H. pylori* infection have only mild asymptomatic gastritis, or develop peptic ulcers and gastric cancer, remains unknown.

The key pathophysiological event in *H. pylori* infection is initiation of an inflammatory response. Cytokines have been suggested to mediate the mucosal inflammation caused by *H. pylori* [4]. *H. pylori* infection is histologically characterized by neutrophil infiltration, and toxic metabolites and lysosomal enzymes released from neutrophils may be responsible for the gastric mucosal injury [5, 6]. Mucosal IL-6 and IL-8 levels have been reported to be increased in dyspeptic patients infected with *H. pylori* [7, 8]. Being a potent chemoattractant for and activator of neutrophils, IL-8 is thought to play a central role in gastric mucosal injury caused by *H. pylori* [8, 9].

Interleukin(IL)-1β is a potent proinflammatory cytokine and is up-regulated in the presence of *H. pylori*. IL-1β is also a potent inhibitor of gastric acid secretion [10]. The gene for IL-1α and IL-1β, and the IL-1 receptor antagonist gene (*IL-1RN*) are all located on the long arm of chromosome 2 [11, 12]. Three diallelic polymorphisms in *IL-1B* have been reported, all representing C-T base transitions at positions −511, −31 and +3954 [13]. Recently, polymorphisms of the *IL-1 B* and *IL-1RN* genes were reported to be associated with gastric cancer risk [14].

We recently reported that the gastric mucosal IL-1β levels of the antrum were significantly higher in genotype *IL-1B*-511C/C than *H. pylori* negative patients [15].

To determine the role of host immune responses in *H. pylori* infection, we examined

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the relationship between gastric mucosal IL-8 levels and histological gastritis in patients with *H. pylori* infection. We also examined the relationship between gastric mucosal IL-8 levels and *IL-1B* polymorphisms in patients with *H. pylori* infection.

PATIENTS AND METHODS

Subjects. Ninety-nine patients (M/F: 56/43, age range; 22-80 years, mean; 50 years) were examined. The study was approved by the Tokai University Hospital Ethics Committee, and informed consent was obtained from all patients. Six biopsy specimens were obtained. *H. pylori* infection was diagnosed by histology and cultivation. Biopsy tissue obtained from the greater curvature of the antrum and the greater curvature of middle body of the stomach were homogenized separately, and mucosal IL-8 levels then measured by ELISA.

Gastric mucosal IL-8 levels

Both antrum and corpus biopsy tissue were homogenized in 1ml of phosphate buffered saline (pH 7.4) using a homogenizer and then centrifuged at 1800 rpm for 10 min. The supernatants were kept at −20°C until the assay. The IL-8 levels in the biopsy supernatants were determined using a sandwich-type IL-8 enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, MN). Protein contents were determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, CA). Results are expressed as pg/mg protein.

Histological evaluation

The biopsy specimens from the greater curvature of the antrum and the greater curvature of the middle body of the stomach were fixed in 10% buffered formalin. The extent of *H. pylori* infection, neutrophil infiltration, mononuclear cell infiltration and atrophy were assessed according to the Updated Sydney system and scored from 0 to 3 [16].

Determination of Gene Polymorphism

Peripheral blood samples were obtained from the 99 patients, and genomic DNA then extracted using a DNA extraction kit (Takara, Otsu, Japan).

Polymorphism

Single-base polymorphisms at positions −511 and −31 in the promoter region of *IL-1B* were analyzed by PCR-RFLP as previously described [15]. The frequencies of *IL-1 B*-511C/C, C/T and TT were 24/99, 53/99, and 22/99, respectively. The frequencies of *IL-1B*-31 C/C, C/T and T/T were 22/99, 53/99, and 24/99 respectively.

Statistical analysis

The Mann-Whitney test was used to compare the data with *H. pylori* infection levels. Multiple comparisons were performed using the Kruskal-Wallis test followed by the Mann-Whitney U-test with Bonferroni correction. The relation between histological findings and the production of IL-1β was assessed by Spearman' rank correlation coefficient.

RESULTS

Gastric mucosal levels of IL-8

The patient profiles are listed in Table 1. *H. pylori* was detected in 64 patients. IL-8 levels in both the antrum and corpus were significantly higher in *H. pylori* positive patients than negative patients (Fig. 1). No difference

Table 1 Characteristics of 99 patients

	H.pylori positive (n=65)	*H.pylori* negative (n=34)
Age	45.0	50.8
Sex M/F	16/18	40/25
Peptic ulcer	21	2
Chronic gastritis	42	4
Gastric carcinoma and adenoma	2	0
Non-ulcer dyspepsia	0	18
Reflux esophagitis	0	2
Post eradication of *H.pylori*	0	8

in IL-8 levels was observed between *H. pylori* negative patients and those who underwent successful eradication of *H. pylori*. The gastric mucosal IL-8 levels in both the antrum and corpus were higher in patients with *H. pylori* than in *H. pylori* negative patients, regardless of *IL-1B* polymorphism (Fig. 2).

IL-8 production and histological findings

The mucosal levels of IL-8 protein significantly correlated with the density of *H. pylori* in both the antrum and corpus (Figs. 3, 4). The mucosal levels of IL-8 in both the antrum and corpus were higher in patients with severe neutrophil and mononuclear cell

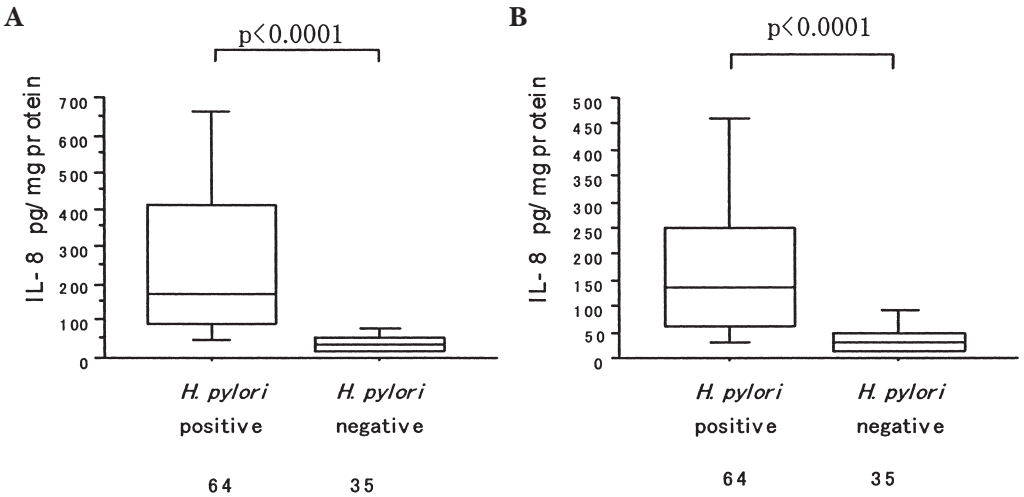

Fig. 1 Gastric mucosal IL-8 levels in patients with *Helicobacter pylori* infection. (**A**) IL-8 levels in the antrum. (**B**) IL-8 levels in the corpus.

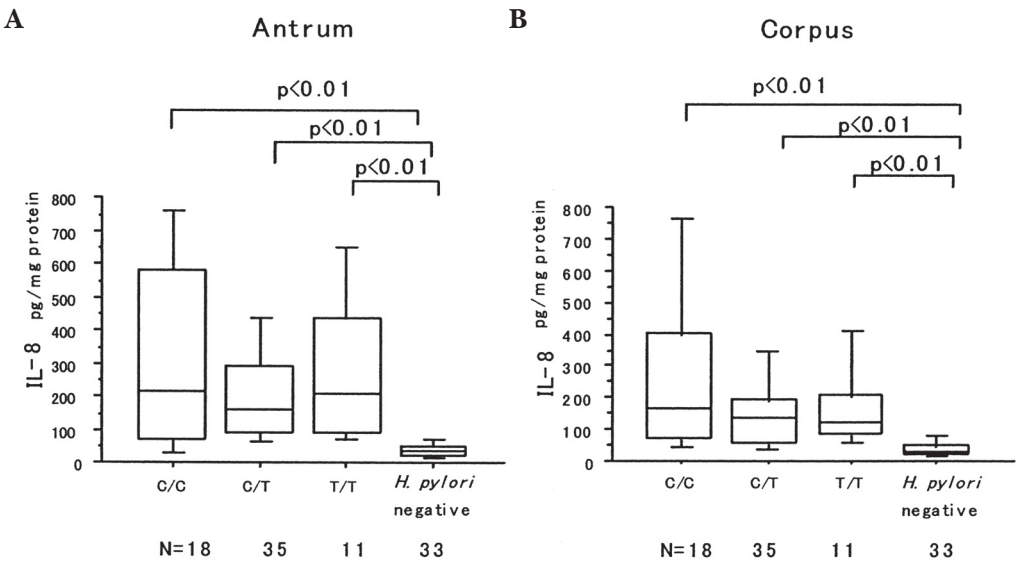

Fig. 2 Gastric mucosal IL-8 levels in the antrum (**A**) and corpus (**B**) in relation to the genotypes at *IL-1B*-511.

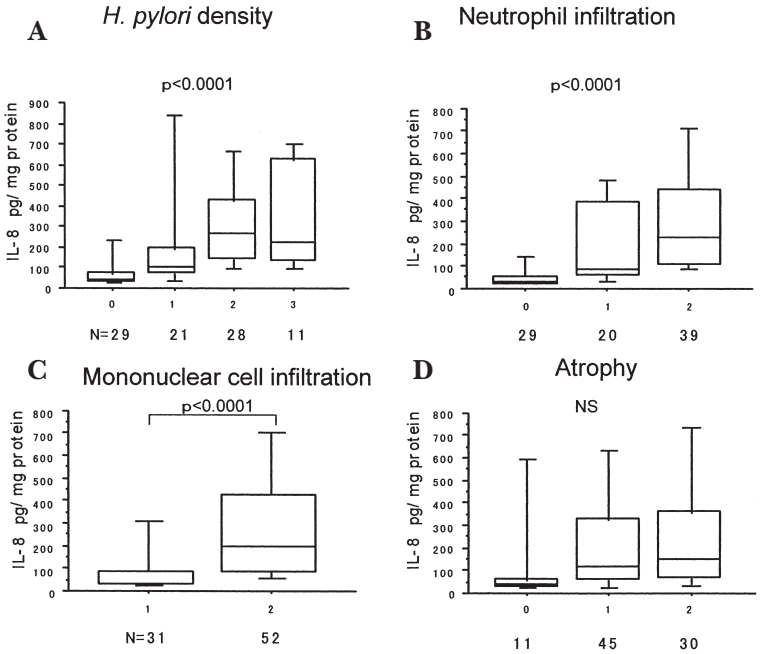

Fig. 3 Relationship between IL-8 and histological gastritis in the antrum. (**A**) *H. pylori* density, (**B**) neutrophil filtration, (**C**) inflammation and (**D**) atrophy with IL-8. (A), (B), (D): calculated by Spearman rank test. (**C**): calculated by the Mann-Whitney test.

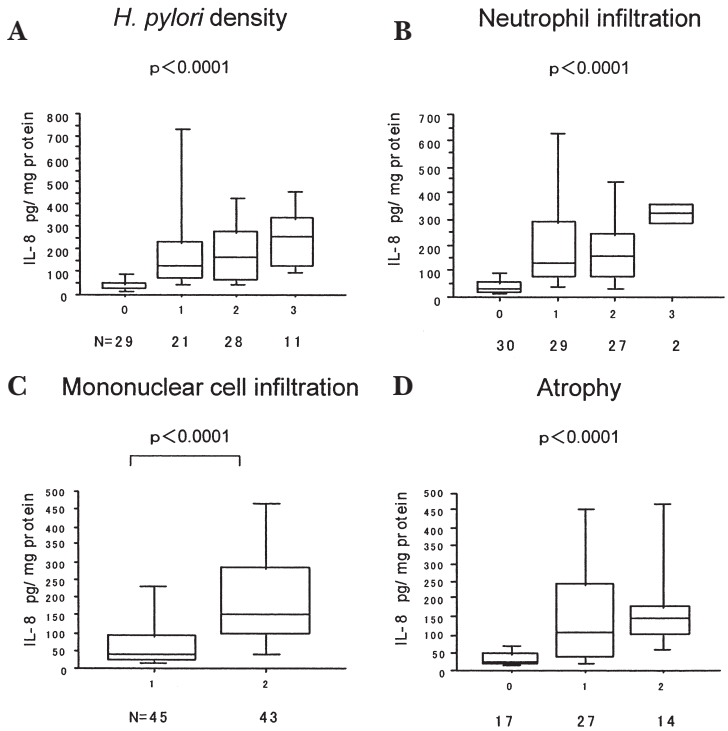

Fig. 4 Relationship between IL-8 and histological gastritis in the corpus. (**A**) *H. pylori* density, (**B**) neutrophil filtration, (**C**) inflammation and (**D**) atrophy with IL-8. (A), (B), (D); caluculated by Spearman rank test. (**C**): caluculated by The Mann-Whitney test.

infiltration than in those without infiltration. The mucosal levels of IL-8 in both the antrum and corpus were higher with severe mononuclear cell infiltration than mild infiltration. Interestingly, IL-8 levels in the corpus but not the antrum correlated with the severity of the atrophy.

DISCUSSION

It is widely accepted that chronic *H. pylori* infection induces hypochlorhydria and gastric atrophy, both of which are precursors of gastric cancer [17]. It has been suggested that cytokines mediate the gastric mucosal inflammation caused by *H. pylori*. Among the inflammatory cytokines, IL-8 may have a major role in *H. pylori* infection, because *H. pylori* infection is histologically characterized by neutrophil infiltration [1]. We confirmed in previous reports that gastric mucosal IL-8 levels are significantly higher in *H. pylori* positive patients than negative patients [7-9]. In the present study, the mucosal levels of IL-8 in both the antrum and corpus were higher with moderate neutrophil and mononuclear infiltration than with no infiltration. Furthermore, IL-8 levels were correlated with gastric atrophy in the corpus but not in the antrum. *H. pylori* infection exerts diverse effects on gastric physiology. The disturbance in acid secretion is related to the pattern of gastritis induced by the infection. In patients with antral-predominant gastritis, acid secretion is normal or increased. This pattern of gastritis is seen in patients with duodenal ulcer. In contrast, a corpus predominant gastritis is associated with reduced acid secretion. A recent prospective study by Uemura *et al.* [19] showed that gastric cancer developed in 36 of 1246 *H. pylori*-infected patients but in none of the 280 uninfected patients. They also reported that among patients with *H. pylori* infection, those with severe gastric atrophy, corpus-predominant gastritis, and intestinal metaplasia, were at a higher risk for gastric cancer.

Recently El-Omar *et al.* reported that *IL-1B* gene polymorphisms (*IL-1B*-511TT) and *IL-1RN**2/2* are associated with an increased risk of hypochlorhydria and gastric cancer [14]. Machado *et al.* also reported that carriers of *IL-1B*-511T and *IL-1RN**2/2* had increased risk for developing intestinal-type gastric cancer with odds ratios of 2.7 and 3.1, respectively [20]. Suppression of acid secretion leads to *H. pylori* re-distribution to the corpus, and hence gastric atrophy [21]. However, conflicting data has been reported regarding the association between *IL-1B* polymorphisms and gastric cancer risk in the Japanese population. Matsukura *et al.* analyzed the polymorphisms and PG I/II ratio in Japanese, Chinese, Thai, and Vietnamese patients [22], and found that the *IL-1B* polymorphisms did not differ among the four populations, but that in cases with severe mucosal atrophy, the C/C polymorphism was dominant in the Japanese population whereas the T/T +T/C polymorphisms were dominant in the Chinese population. Furthermore, Kato *et al.* [23] reported no association between *IL-1B* specific genotypes and gastric cancer in the Japanese population. This discrepancy may be explained by the difference in haplotype frequencies of *IL-1B* and *IL-1RN* gene polymorphisms between the Japanese population and Western populations. Machado *et al.* reported that the frequency of the *IL-1RN**2 allele in their controls and gastric cancer subjects was 20/220 and 24/152, respectively [20]. In contrast, Hamajima *et al.* [24] reported that the frequency of *IL-1RN*2*2* was 1/ 241 cases in Japanese subjects. Thus, *IL-1RN* may play a more important role in the development of gastric cancer in Western countries than in Japan.

Since IL-1β is a potent inhibitor of gastric acid secretion, IL-1β may enhance IL-8 production through re-distribution of *H. pylori*. Yamaoka *et al.* reported the mucosal level of IL-8 is correlated with that of IL-1β [25]. Therefore, we analyzed the relationship between *IL-1B* polymorphism and IL-8 production. We found that the mucosal levels of IL-8 in both the antrum and corpus were higher in patients with severe neutrophil and severe mononuclear cell infiltration than in those without infiltration. However, IL-8 levels of the corpus were higher in *H. pylori* positive patients than *H. pylori* negative patients, regardless of *IL-1B* polymorphism. Furthermore, the polymorphism did not influence the density of *H. pylori* and gastric atrophy [15]. The reason for this discrepancy is unclear, although one possible explanation is the difference in composition between two study populations. Furthermore, IL-1β levels may be influenced by other than *IL-1B*

gene polymorphism. These findings provide further evidence of no association between *IL-1B* specific genotype and gastric cancer in the Japanese population.

In conclusion, *IL-1B* polymorphisms enhance IL-1β production, however IL-8 production is independent of *IL-1B* polymorphism, and both cytokines may play an important role in the development of gastric mucosal inflammation. IL-8 appears to play a major role in the development of atrophic gastritis.

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