A M_r 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*

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The complete genome sequence revealed a family of 32 outer membrane proteins (OMPs) in *Helicobacter pylori.* We examined the effect of four OMPs (HP0638, HP0796, HP1501, and *babA2*) on the production of the proinflammatory cytokine, IL-8. Mutants of the four OMPs, as well as *cagE* and *galE* from *H. pylori* from the U.S. and Japan, were constructed by inserting a chloramphenicol-resistant cassette into the gene. Twenty-two pairs of parental and mutant *H. pylori* strains, as well as 160 clinical isolates (80 from Japanese and 80 from U.S.), were cocultured with gastric cancer cell lines. IL-8 production in the supernatant and adhesion was assayed by ELISA. HP0796, HP1501, *babA2*, and *galE* gene knockouts had no significant effect on IL-8 production. Knockout of the HP0638 gene in 81% of *cag*-positive strains reduced IL-8 production approximately 50%. The three *cag*-positive strains in which IL-8 levels were unchanged by HP0638 knockout had five or seven CT dinucleotide repeats in the 5′ region, resulting in a frame shift and truncation. Strains with naturally inactive HP0638 gene were all from the U.S.; Japanese strains were always ''on'' and thus, on average, may be more virulent. Although *cag*-negative isolates produced a limited IL-8 response, *cag*-negative strains that contained a functional HP0638 gene produced more than 3-fold greater IL-8 than *cag*-negative nonfunctional HP0638 strains. We hypothesize that functional HP0638 gene may be an important virulence factor in relation to the risk of clinically significant outcomes of *H. pylori* infection. We denote HP0638 gene as outer inflammatory protein (*oipA*).

Helicobacter pylori-related gastritis is characterized by mucosal infiltration with neutrophils and mononuclear cells. The migration and activation of these cells into the gastric mucosa is thought to be related to the local production of various proinflammatory cytokines (1, 2), especially IL-8, a potent neutrophil chemotactic and activating peptide (3). Antral mucosal IL-8 levels are closely related to the *H. pylori* density (2), and *H. pylori* density, cellular infiltration, and mucosal IL-8 levels are significantly greater in duodenal (DU) ulcer than in simple gastritis, suggesting a role for IL-8 in *H. pylori* inflammation and *H. pylori*-associated gastroduodenal diseases (2).

Studies regarding the relationship between *H. pylori* and IL-8 have primarily focused on the *cag* pathogenicity island (PAI) (1, 2, 4–6). The presence of a functional *cag* PAI is associated with increased IL-8 production, and many of the genes contained within this island, such as *cagE*, may affect the inflammatory response (6). However, there are also data to suggest that the *cag* PAI is not the sole *H. pylori* factor able to promote IL-8 secretion. We, and others, have found that some *cag*-negative strains produced IL-8 from cell lines such as MKN45, AGS, and KATO III (2, 4, 5). In addition, although IL-8 levels are typically low in gastric biopsy specimens from patients with *cag*-negative *H. pylori* infections, mucosal IL-8 levels in some *cag*-negative cases are higher than the median IL-8 values of *cag*-positive cases and showed severe cellular infiltration (2). Together, these observations suggest the presence of a virulence factor(s) other than the *cag* PAI involved in IL-8 production.

In vitro experiments indicate that IL-8 is produced from the epithelial cells after viable *H. pylori* attached to the cells (5). Thus, whereas direct interaction and possibly translocation of factors are known to be important (7), the outer membrane proteins (OMPs) are good candidates for the unknown proinflammatory virulence factor(s). Thirty-two genes encoding OMPs were identified after computer inspection of two complete genome sequences of *H. pylori* (8, 9).

We recently reported that the IL-8 levels in the corporal mucosa were related to the presence of serum Ab against a M_r 33,000 to 35,000 Ag (10). Only two gene products, HP0638 (M_r 34,000) and HP0796 (M_r 33,000), were within that range of molecular weight among the OMP family (HP number from ref. 8). We had previously studied a M_r 32,000 Ag from *Mycobacterium paratuberculosis,* which had binding properties to fibronectin and may be an adherence factor (11). Using primers specific for this Ag, the same size fragment was amplified from *H. pylori* DNA. Sequence analysis indicated that this fragment was from an OMP (HP1501).

In this study, we examined the roles of four OMPs (HP0638, HP0796, HP1501, and BabA) in IL-8 induction. We chose BabA as it has been described as an OMP that appears to be involved in adherence of *H. pylori* to Lewis-b (Le^b) blood group Ags on gastric epithelial cells (12). Functional BabA is encoded by the *babA2* gene. The *babA2* gene is identical to the *babA1* gene with the exception of an insert of 10 bp with a repeat motif in the signal peptide sequence, which results in the creation of a translational initiation codon and has Le^b binding ability. We also examined the *galE* gene, which is involved in lipopolysaccharide biosynthesis of *H. pylori* (13) as a control and as a candidate to induce IL-8. Here we show that the functional HP0638 gene may be an important virulence factor in relation to the risk of clinically significant outcomes of *H. pylori* infection. We designated HP0638 gene as outer inflammatory protein (*oipA*) gene of *H. pylori*.

Materials and Methods

***H. pylori* Studied.** *H. pylori* were obtained from 80 Japanese patients (56 men and 24 women; mean age 52 years) from Kyoto Prefectural University of Medicine (Kyoto, Japan) and 80 U.S. patients (67 men and 13 women; mean age 52 years) from Veterans Affairs Medical Center (Houston, TX) (40 with DU, and 40 with gastritis in both countries). DU was identified endoscopically, and gastritis was defined as histologic gastritis with no peptic ulcer, gastric cancer, or esophageal disease.

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Abbreviations: OMP, outer membrane protein; PAI, pathogenicity island; DU, duodenal ulcer; *oipA*, outer inflammatory protein.

Data deposition: The DNA sequences reported in this paper have been deposited in the GenBank database (accession nos. AF233660–AF233683).

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Biopsies were obtained with informed consent from all patients under protocols approved by each local ethics committee.

In addition, *H. pylori* 26695 (ATCC 700392) (American Type Culture Collection, Manassas, VA) and Sydney strain (SS1) (a gift from A. Lee, University of New South Wales, Sydney, Australia) were used as reference *H. pylori* strains.

***H. pylori* Genotyping.** Antral biopsy specimens were obtained for isolation of *H. pylori,* as previously described (14). Multiple colonies were collected together, and all stock cultures were maintained at −80°C in Brucella broth supplemented with 20% glycerol. Clinical isolates underwent a maximum of seven *in vitro* passages before incubation with the gastric epithelial cells. The two reference strains (26695 and SS1) had been passaged multiple times without documentation of the exact number of passages. Genomic DNA from *H. pylori* was extracted by using the QIAamp Tissue kit (Qiagen, Chatsworth, CA).

cag PAI status was evaluated by PCR for the combination of *cagA, cagE*, and *cagG* and by immunoblot analysis for CagA protein by using recombinant CagA polyclonal Ab (Oravax, Cambridge MA), as previously described (2). The *vacA* genotype (s and m region), *iceA* allele (*iceA1* or *iceA2*), and *babA2* status (*babA2*-positive or -negative) were evaluated by PCR, as previously described (14, 15).

Construction of Isogenic Mutant Strains of *H. pylori*. For construction of isogenic mutant strains, we collected a single colony from stock frozen *H. pylori* that had been collected from multiple colonies. A portion of the genes encoding HP0638, HP0796, HP1501, *babA2*, and *cagE* gene were amplified by PCR, and the amplified-fragment was inserted into the *Eco*RV restriction enzyme site of pBluescriptSK+ (Stratagene). A chloramphenicol resistance gene cassette (*cat*) (a gift from D. E. Taylor, University of Alberta, Edmonton, Canada) was inserted into *Ssp*I, *Eco*47III, *Acc*I, *Bgl*II, and *Eco*47III sites of the insert DNA for HP0638, HP0796, HP1501, *babA2*, and *cagE*, respectively. A kanamycin resistance gene cassette (*km*) (a gift from R. Haas, Max von Pettenkofer Institut, Munich, Germany) was also inserted into the *Eco*47III site of insert DNA for *cagE*, and the resulting plasmid was used for dual inactivation by selecting on a chloramphenicol and kanamycin plate. All of the plasmids (1 to 2 μg) were used for inactivation of chromosomal genes by natural transformation as previously described (16). Inactivation of the genes was confirmed by PCR amplification followed by Southern blot hybridization. Isogenic *galE* mutant was constructed as previously described (13).

IL-8 Levels from Gastric Cancer Cells Cocultured with *H. pylori*. *In vitro* IL-8 measurement was performed as previously described (2). Briefly, MKN45 cells (1×10^5/well), AGS cells (5×10^4/well), and KATO III cells (1×10^5/well) were plated into 24-well plates and cultured for 2 days (about 5×10^5/ml for each cells). Stock frozen *H. pylori* was cultured in brain–heart infusion broth containing 5% horse serum with a rotatory shaker for 24 to 48 h, representing growth phases. Isogenic mutants of each gene were used for incubation with the same *in vitro* passage level as parental strains. *H. pylori* was added to the cultured cells (bacterium-to-cell ratio of 100:1) and incubated for 24 h. IL-8 in the supernatant was assayed by ELISA (R&D Systems) in duplicate for experiments that used only wild-type strains or in triplicate for experiments that used parental and knockout mutants.

Sequences Analysis of the HP0638 Gene. According to the complete genome sequence of *H. pylori*, dinucleotide repeats were located in the region encoding the signal sequences of HP0638 (8, 9). The signal sequences of HP0638 including the repeats were amplified by PCR using primer pairs 5′-CAAGCGCTTAACAGATAGGC-3′ (forward) and 5′-GCTTCACGAGAAAACGCCTT-3′ (reverse). Amplified DNA fragments were subcloned into the pBluescriptSK+ (Stratagene), and the DNA sequence determination was performed at the Molecular Genetics Facility at Baylor College of Medicine.

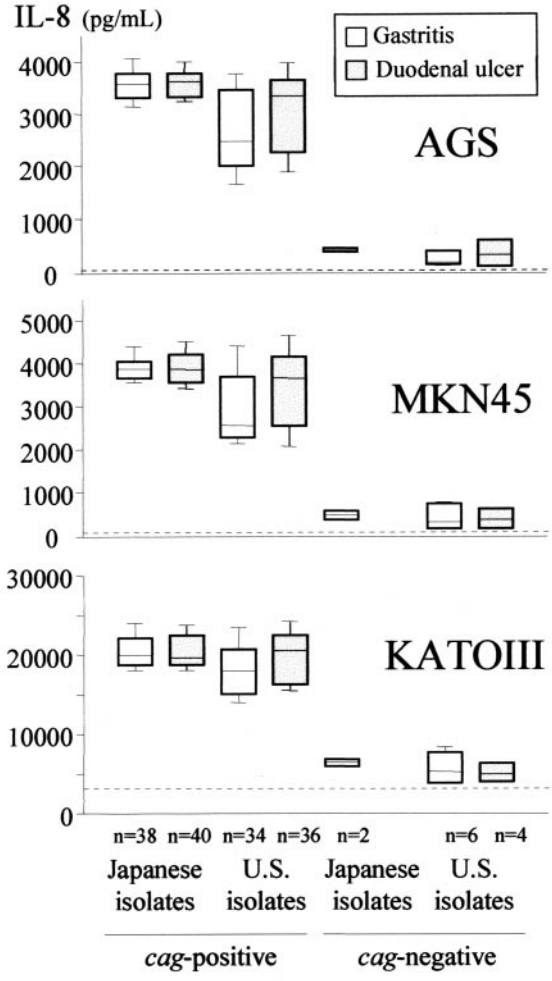

Fig. 1. IL-8 production from three gastric cancer cell lines cocultured with clinical isolates of *H. pylori* from Japanese and U.S. patients. *H. pylori* was added to the cultured cells (bacterium-to-cell ratio of 100:1) for 24 h, and IL-8 in the supernatant was assayed by ELISA. The broken line indicates the IL-8 levels from each cancer cell without being cocultured with *H. pylori* (control). The ends of the bars indicates the 25th and 75th percentiles. The 50th percentile (median) is indicated with a line in the bar, and the 10th and 90th percentiles are indicated with error bars.

Quantification of *H. pylori* Adhesion to Gastric Cancer Cells. Quantitative evaluation of *H. pylori* adhesion to AGS cells was performed by ELISA as previously described (17, 18). Briefly, AGS cells (5×10^4/well) were plated into 96-well plates and cultured for 2 days (about 5×10^5/well). *H. pylori* (the culture conditions were identical to those in the experiments for IL-8 measurement) was added to the cultured cells (bacterium-to-cell ratio of 500:1) and incubated for 90 min. After washing, adherent *H. pylori* and cells were fixed at 4°C for 60 min by using 8% paraformaldehyde. Anti-*H. pylori* Ab (diluted 1:50) (Dako) was used for the first Ab and peroxidase-conjugated goat anti-rabbit immunoglobulins (diluted 1:1000) (Sigma) for the second Ab; *o*-phenylenediamine (0.4 mg/mg) (Sigma) was the substrate. The reaction was terminated by 3 M H2SO4, and the OD at 490 nm

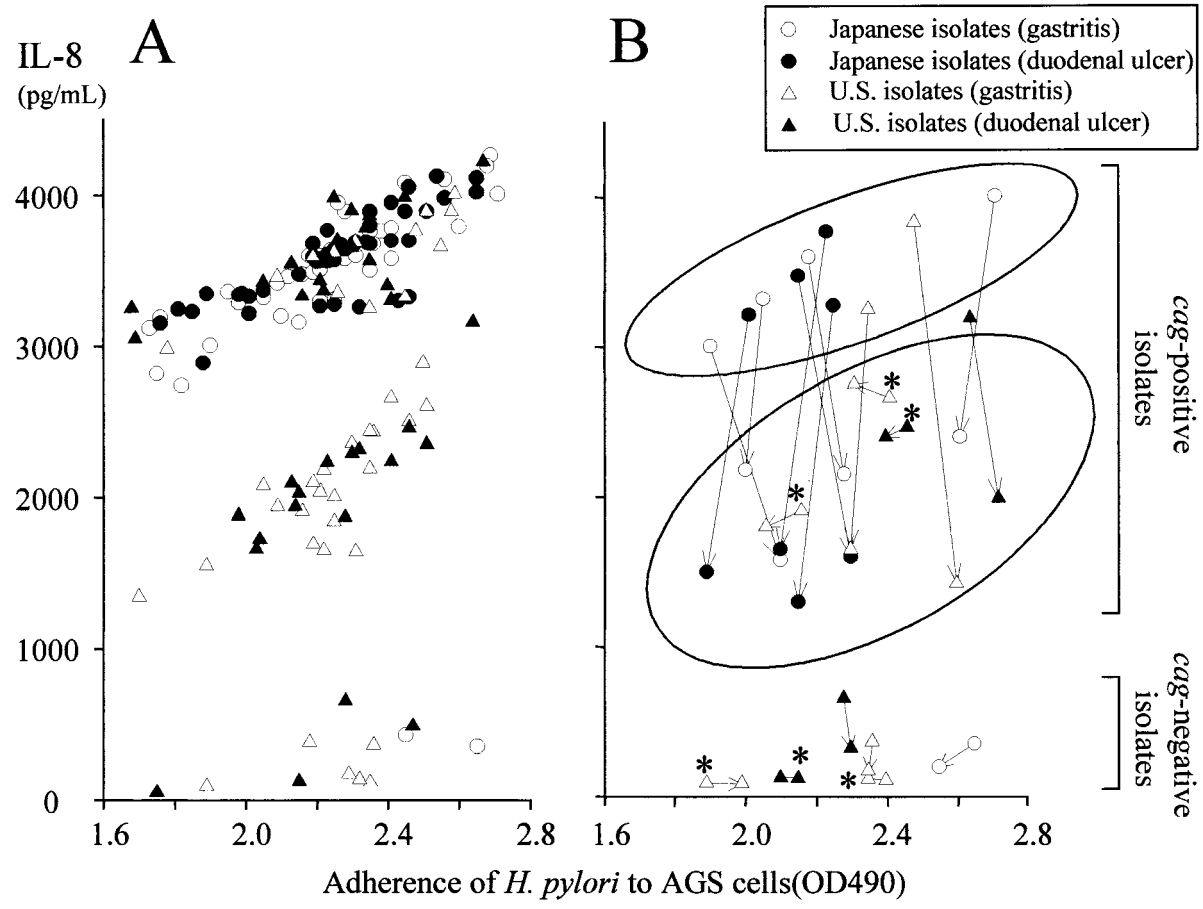

Fig. 2. Relation between adherence of *H. pylori* (OD_{490}) to AGS cells and IL-8 production from AGS cells cocultured with *H. pylori* clinical isolates (*A* and *B*) and *oipA* knockout mutants (*B*). *H. pylori* was added to the cultured cells (bacterium-to-cell ratio of 100:1) for 24 h, and IL-8 in the supernatant was assayed by ELISA. *H. pylori* was added to the cultured cells (bacterium-to-cell ratio of 500:1) for 90 min, adherent *H. pylori* and cells were fixed at 4°C for 60 min, and adherence was assayed by ELISA using anti-*H. pylori* Ab (diluted 1:50) as a first Ab. The OD at 490 nm was used as the index of the number of *H. pylori* adhering to AGS cells. In *B*, the beginning of the array shows wild-type strains, and the end of the array shows *oipA* knockout mutants. The * indicates that the strains have nonfunctional *oipA*; therefore, the *oipA* knockout had no effect on IL-8 production.

was used as the index of the number of *H. pylori* adhering to AGS cells (17).

Data Analysis. Statistical analysis was performed by Mann–Whitney Rank Sum test, paired *t*, and Spearman rank test depending on the data set of concern. A P value of less than 0.05 was accepted as statistically significant.

Results

IL-8 Production from Gastric Cancer Cells Cocultured with Wild-Type *H. pylori*. *H. pylori* had the ability to induce IL-8 from all three cancer cell lines (Fig. 1). Although IL-8 secretion differed according to the cell lines used, the IL-8 levels were significantly related among the cancer cells (AGS vs. MKN45; $r = 0.91$, AGS vs. KATO III; $r = 0.89$, MKN45 vs. KATO III, $r = 0.89$; $P < 0.0001$ for each). Therefore, we present detailed data using AGS cells.

There was no relationship between IL-8 levels and the *iceA* allele either from U.S. or Japanese strains (U.S., 2309 and 2549 pg/ml; Japanese, 3523 and 3222 pg/ml; *iceA1* and *iceA2*, respectively). High IL-8 production was observed in *cag*-positive strains irrespective of whether *vacA* s or m subtype or *babA2* status (the mean IL-8 levels in *vacA* s2, *vacA* m2, and *babA2*-negative were 3362, 3175, and 3325 pg/ml, respectively) compared with 3231, 3238, and 3228 pg/ml for *vacA* s1, *vacA* m1, and *babA2*-positive strains, respectively. In addition, low IL-8 production was observed in *cag*-negative strains irrespective of *vacA* s or m subtype or *babA2* status (the mean IL-8 level in *vacA* s1, *vacA* m1, or *babA2*-positive strains was 158, 394, and 135 pg/ml, respectively). Together, these findings are consistent with the notion that IL-8 production is most dependent on a functional *cag* secretion system and less on *vacA* or *babA* status.

Even among *cag*-positive isolates, IL-8 levels differed greatly, especially among the U.S. isolates (Fig. 1). IL-8 levels among the *cag*-positive Japanese isolates were more homogenous and significantly greater than with *cag*-positive U.S. isolates (Japanese, 3566 pg/ml; U.S., 2862 pg/ml; $P < 0.0001$). In the U.S. *cag*-positive isolates, IL-8 levels were significantly higher in DU cases compared with isolates from gastritis (DU, 3052 pg/ml; gastritis, 2662 pg/ml; $P < 0.05$). There was no difference in IL-8 levels among Japanese isolates irrespective of clinical presentation.

Adherence to Gastric Cancer Cells with Wild-Type Clinical Strains. There was no relationship between adherence and *cag* PAI (Fig. 2*A*), *babA2*, *iceA*, or *vacA* genotypes (data not shown). Among *cag*-positive isolates, there was a significant relationship between adherence and IL-8 production from the AGS cells (Japanese strains, $r = 0.83$; U.S. strains, $r = 0.48$; $P < 0.0001$ for each) (Fig. 2*A*). The U.S. *cag*-positive isolates could be clearly divided into two groups. One overlapped the Japanese isolates; the other

Table 1. Data regarding the parental strains used for constructing isogenic mutants

Strain	Country	Disease	*cag* PAI	*vacA*	*iceA*	*babA2*
26695	U.K.	Gastritis	+	s1b-m1	1	−*
SS1	Australia	Gastritis	+	s2-m2	2	−
JK43, JK44, JK46, JK51	Japan	Gastritis	+	s1c-m1	1	+
JK23, JK91	Japan	DU	+	s1c-m1	1	+
JK25	Japan	DU	+	s1c-m1	2	−
JK35	Japan	DU	+	s1c-m1	2	+
GI2060, GI2777	U.S.	Gastritis	+	s1b-m1	2	+
GI2895	U.S.	Gastritis	+	s1a-m1	1	+
GI2975	U.S.	Gastritis	+	s1b-m1	1	+
GI2450	U.S.	DU	+	s1b-m2	2	+
GI2826	U.S.	DU	+	s1a-m2	2	+
JK2-41	Japan	Gastritis	−	s1c-m1	2	+
GI2853	U.S.	Gastritis	−	s2-m2	2	+
GI2924	U.S.	Gastritis	−	s2-m2	2	−
GI3009	U.S.	Gastritis	−	s2-m2	1	−
GI2490, GI2685	U.S.	DU	−	s2-m2	2	−

**H. pylori* 26695 had reported as *babA1* genotype because this has no ability to bind to Lewis b Ag. However, 26695 was classified as *babA2* by our PCR techniques because sequence analysis of this strain showed that there was a 10-bp repeat motif in *babA* gene (HP1243).

showed a positive correlation but with lower IL-8 production, suggesting that a mechanism other than adherence might be involved in IL-8 induction.

Generation of Isogenic Mutant Strains of *H. pylori*. To evaluate the natural competence and transformation ability of *H. pylori* strains, we selected 40 clinical isolates (20 Japanese isolates and 20 U.S. isolates), as well as *H. pylori* 26695 and SS1. As a control gene for checking natural transformation, we chose the *galE* gene as it is not essential for *H. pylori* survival (13). Twenty-two strains (9 Japanese isolates, 11 U.S. isolates, 26695, and SS1) were transformable and were used for constructing gene negative mutants for HP0638, HP0796, HP1501, *babA2*, and *cagE* gene (Table 1).

Effect of Isogenic Mutant Strains for IL-8 Production and Adherence to Cells. There was no difference in the ability to promote IL-8 secretion between the wild-type and isogenic HP1501, *babA2*, and *galE* mutants (data not shown). The HP0796 knockout mutant showed a minor reduction of IL-8 (mean 13% reduction). In contrast, HP0638 and *cagE* knockout mutants showed a major reduction in IL-8 production (mean 40% reduction) (Fig. 3). We designated HP0638 gene as outer inflammatory protein (*oipA*) of *H. pylori* because IL-8 has a major inflammatory effect in the gastric mucosa.

There were two types of *oipA* knockout mutants of *cag*-positive isolates in relation to IL-8 induction (Figs. 2*B* and 3*A*). In 13 of 16 cag-positive strains (all 8 Japanese, 3 of 6 U.S., 26695, and SS1), IL-8 production was significantly reduced with the

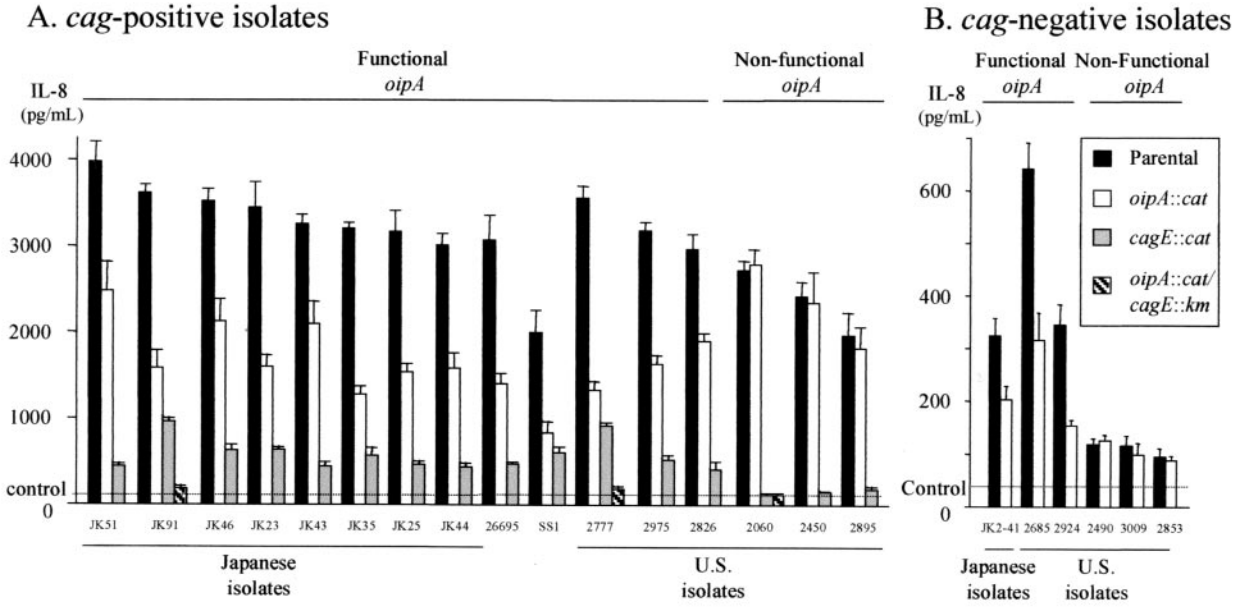

Fig. 3. IL-8 production from AGS cells cocultured with 16 *cag*-positive clinical isolates (*A*) and 6 *cag*-negative clinical isolates (*B*) from Japanese and U.S. patients and their *oipA* or *cagE* knockout mutants. *H. pylori* was added to the cultured cells (bacterium-to-cell ratio of 100:1) for 24 h, and IL-8 in the supernatant was assayed in triplicate by ELISA. Error bars indicate mean + SD. The broken line indicates the IL-8 levels from AGS cells without being cocultured with *H. pylori* (control).

Table 2. Signal-sequence coding region of the *oipA* gene of different *H. pylori* strains

Strains	Sequence of the signal-peptide coding region	Number of CT repeats	Gene status
GI2060	ATGAAAAAAGCCCTCTTACTAACTCTCTCTCT--------CGTTTTGGCTCCACGCTGA	5	Off
	M K K A L L L T L S L V L A P R *		
GI2826	ATGAAAAAAGCCCTCTTACTAACTCTCTCTCTCT------CGTTCTGGCTCCACGCTGAAAGGAATGGATTTTATTTAG	6	On
	M K K A L L L T L S L S F W L H A E R N G F Y L		
GI2895	ATGAAAAAAGCCCTCTTACTAACTCTCTCTCTCTCT----CGTTTTGGCTCCACGCTGAAAGGAACGGATTTTATTTAG	7	Off
	M K K A L L L T L S L S R F G S T L K G T D F I *		
GI2924	ATGAAAAAAGCCCTCTTACTAACTCTCTCTCTCTCTCTCTCTCGTTTTGGCTCCACGCTGAAAGGAACGGATTTTATTTAG	9	On
	M K K A L L L T L S L S L S F W L H A E R N G F Y L		
JK44	ATGAAAAAAGCTCTCTTACTAACTCTCTTTTCT------CGTTTTGGCTCCACGCTGAAAGGAATGGATTTTATTTAG	3+1	On
	M K K A L L L T L F F S F W L H A E R N G F Y L		
JK2-41	ATGAAAAAAACCCTTTTACTCTTTCTCTCTTTCT------CGTTTTGGCTCCACGCTGAAAGGAATGGATTTTATTTAG	3+1	On
	M K K T L L L F L S F S F W L H A E R N G F Y L		
JK51	ATGAAAAAAACCCTTTTACTCTTTCTGCCTTTCT------CGTTTTGGCTCCACGCTGAAAGGAATGGATTTTATTTAG	none	On
	M K K T L L L F L P F S F W L H A E R N G F Y L		

Nucleotide and deduced amino acid sequences of the signal peptide-coding region of the *oipA* gene of strains used for knockout mutant of the *oipA* gene. Asterisks denote the stop codon. The sequence data for all strains examined in this study are available in the GenBank database (AF233660–AF233683).

oipA knockout mutants (mean 49% reduction), which we designated as putative functional *oipA*. In contrast, *oipA* knockout had no effect on IL-8 production in 3 U.S. strains (putative nonfunctional *oipA*).

IL-8 production was low in *cag*-negative parental strains; however, even in *cag*-negative isolates, a different effect of *oipA* knockout was seen between the putative functional and nonfunctional types (Fig. 3*B*) because IL-8 production was significantly reduced (mean 53%) in the putative functional *oipA* knockout mutants compared with their parental strains. The parental *cag*-negative strains with a putative functional *oipA* gene produced more than three times the amount of IL-8 compared with *cag*-negative isolates with a nonfunctional *oipA* gene.

As has previously been reported (4–6), IL-8 production from gastric cancer cells cocultured with the *cagE* knockout mutant was significantly reduced (mean 87% reduction) (Fig. 3*A*). The strains could be divided into two groups in relation to the *oipA* knockout mutants. Knockout of *cagE* of strains in which IL-8 levels were unchanged by *oipA* knockout had almost complete inhibition of IL-8 induction. In contrast, knockout of *cagE* in strains in which IL-8 levels were decreased by *oipA* knockout had only partial reduction in IL-8 production. We therefore constructed double knockout mutants of *oipA* and *cagE* gene from strains with a putative functional *oipA,* JK91, and GI2777, and from GI2060, which has a putative nonfunctional *oipA.* Double knockout of *oipA* and *cagE* reduced IL-8 production almost to control levels in strains with a putative functional *oipA* (90% and 92% reduction, JK91 and G2777, respectively) (Fig. 3*A*) and had no additional effect in strains with a nonfunctional *oipA* (*cagE*::*cat* 96%, and *oipA*::*cat*/*cagE*::*km* 95% reduction). These findings are consistent with the notion that a putative functional *oipA* had a role in IL-8 induction. Knockout mutants of *oipA*, HP0796, HP1501, *babA2, cagE*, or *galE* had no effect on the adherence to AGS cells (data not shown).

Sequence Analysis in the Signal-Peptide Coding Region of the *oipA* Gene. Strains with a putative nonfunctional *oipA* gene contained five and seven CT dinucleotide repeats in the signal-peptide coding region of the *oipA* gene that kept the peptide out-of-frame and the status was "off" (Table 2). In contrast, the U.S. strains that had putative functional *oipA* gene contained six and nine CT dinucleotide repeats that kept the peptide in frame and the status was "on," suggesting that the slipped-strand repair mechanism modulates this gene. All Japanese isolates had only unmatured CT dinucleotide repeats, and the status was always "on".

Interestingly, all isolates with nonfunctional *oipA* gene (status "off") were among those with relatively low IL-8 production compared with adhesion (Fig. 2*B*). In contrast, all but one clinical isolate with a functional *oipA* gene (status "on") had relatively high IL-8 production compared with adhesion.

Effect of Multiple Laboratory Passage in Wild-Type Strains. The maximum passage number of the *H. pylori* isolates used was seven (except for 26695 and SS1). To examine the effect of *in vitro* passage, IL-8 production from cancer cells cocultured with *H. pylori* with different passage numbers [initial passage (3 to 5), 20, and 30 passages] was measured by using five clinical isolates (three U.S. and two Japanese strains) obtained from a single colony. IL-8 levels were decreased dramatically after 30 passages in one U.S. isolate (mean ± SD, initially 3620 ± 346; and after 30 passages, 1543 ± 254 pg/ml; $P < 0.0001$). Sequence analysis showed that the number of CT dinucleotide repeats in the *oipA* gene had changed from six (status "on") (initial) to five (status "off") (30 passages). In four other clinical isolates, IL-8 levels decreased slightly after 30 passages (initially 3457 ± 487; and after 30 passages, 3050 ± 367 pg/ml; $P < 0.05$). There were no changes in the number of CT dinucleotide repeats in the *oipA* gene in these four isolates, indicating that some unknown factors induced by multiple passages may be involved in inhibition of IL-8 *in vitro*. Adherence to AGS cells was not changed by multiple passage in all five strains (data not shown).

Discussion

We found that not only *cag* PAI but also the *oipA* gene has the ability to induce IL-8 from gastric epithelial cells. Three other members of the OMP family, *babA*, HP0796, and HP1501, had no significant effect on IL-8 production. *H. pylori* could be divided into two types (functional and nonfunctional) in relation to the *oipA* gene, based on their ability to induce IL-8. Inactivation of the functional *oipA* gene caused an approximately 50% reduction in IL-8 production. Inactivation of the *cagE* gene caused a dramatic reduction in IL-8 production; IL-8 secretion

remained significantly above control values if the parental strain had a functional *oipA* gene. Double knockout of the *cagE* and *oipA* genes almost completely eliminated IL-8 production. Spontaneous natural inactivation of the *oipA* gene that occurred during multiple passage *in vitro* was also associated with a marked reduction of IL-8 production. Together, these findings suggest that a functional *oipA* gene plays an important role in IL-8 induction.

We also showed that the *oipA* gene is regulated by slipped-strand repair mechanism. Based on the complete genome sequence of *H. pylori*, five members of the OMP family contain CT dinucleotide repeats in their signal-sequence coding regions (8, 9). Recently, another member of the OMP family, *hopZ* gene, whose product was related to adherence, was also reported to be regulated by the slipped-strand repair mechanism (19). Together, these findings support the hypothesis that slipped-strand repair mechanisms have evolved in bacterial pathogens to increase the frequency of phenotypic variation in genes involved in critical interactions with their hosts (8, 9).

Japanese clinical isolates had greater ability to induce IL-8 compared with the U.S. isolates, and this ability was associated with the fact that there were only unmatured CT dinucleotide repeats in the *oipA* gene in the Japanese isolates, such that the status was always ''on.'' The difference in the ability to induce IL-8 among Japanese and U.S. isolates disappeared when we compared only isolates with a functional *oipA* gene. It is tempting to speculate that the different prevalence in gastroduodenal diseases between Japan and the U.S. may be, in part, related to the difference in the prevalence of *H. pylori* with a functional *oipA* gene. *In vitro* IL-8 levels were significantly higher in DU isolates in the U.S. compared with gastritis isolates. There may be relationship in the different prevalence of functional *oipA* gene between DU and gastritis.

The exact mechanism of *H. pylori*-associated IL-8 production from the epithelial cells has not been established. It is now recognized that CagA is translocated into the host cell by the type IV secretion system and becomes tyrosine-phosphorylated (20–23). However, the *cagA* gene knockout mutant does not affect IL-8 production *in vitro* (4–6). Several investigators studying the signal transduction pathway related to *H. pylori*-associated IL-8 induction have noted that *H. pylori* differ in their ability to promote IL-8 secretion (4–6). These differences may have reflected undetected differences in the *oipA* gene. Further study will be necessary to investigate the relationship between the *oipA* gene and *H. pylori*-associated induction of IL-8 secretion.

With a few exceptions, IL-8 production *in vitro* requires the presence of attachment of viable *H. pylori* to the epithelial cells (5). Although neither *oipA* nor *cagE* knockout had an effect on adherence, we found a positive correlation between adherence and IL-8 levels in strains with a functional as well as a nonfunctional *oipA* gene, suggesting that, although adherence is required, the *oipA* gene products role in IL-8 induction is related to a mechanism that is independent of adherence. *H. pylori* are reported to use at least five different adhesins to attach to gastric epithelial cells (8), suggesting that knockout mutants of only one of these genes may not reduce adherence to gastric cells and that multiple number of OMP families may cooperate in adhering to the epithelial cells.

We initially selected HP0638 (*oipA)* (M_r 34,000) and HP0796 (M_r 33,000) because of our observation that IL-8 levels in biopsies of the gastric corpus in Japanese patients were related to the presence of serum Ab against a M_r 33,000 to 35,000 Ag (10). However, all of the Japanese strains had a functional *oipA* gene, suggesting that the M_r 33,000 to 35,000 Ag noted in our previous study may be different from the *oipA* gene.

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