Correction

MICROBIOLOGY. For the article “A M, 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori” by Yoshio Yamaoka, Dong H. Kwon, and David Y. Graham, which appeared in number 13, June, 20, 2000, of Proc. Natl. Acad. Sci. USA (97, 7533–7538), the authors note the following correction. The sequence of the HP0638 gene was incorrect as published. The sequence published was 5′-GCTTCACGAGAAAACGC-CTT-3′ (reverse). The correct sequence is 5′-AAGGCGTTTT-TCTGCTGAAGC-3′ (reverse).

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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. Knockout of the HP0638 gene 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.

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.

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 withDU, 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.

This paper was submitted directly (Track II) to the PNAS office.

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 EcoRV 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 SspI, Eco47III, AccI, BglII, and Eco47III 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 Eco47III 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 × 10⁵/well), AGS cells (5 × 10⁴/well), and KATO III cells (1 × 10⁵/well) were plated into 24-well plates and cultured for 2 days (about 5 × 10⁵/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 (bacterium-to-cell ratio of 100:1) and incubated for 24 h. IL-8 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 (R&D Systems). 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).

**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 × 10⁴/well) were plated into 96-well plates and cultured for 2 days (about 5 × 10⁵/ml) for 24 h. *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/ml) (Sigma) was the substrate. The reaction was terminated by 3 M H₂SO₄, and the OD at 490 nm.
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 level 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. 2A), *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. 2A). The U.S. *cag*-positive isolates could be clearly divided into two groups. One overlapped the Japanese isolates; the other

![Fig. 2. Relation between adherence of *H. pylori* (OD490) 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.](https://www.pnas.org/content/97/13/7535)
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. 2B and 3A). 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

![Graph](https://www.pnas.org)  
*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 1. Data regarding the parental strains used for constructing isogenic mutants

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

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

<table>
<thead>
<tr>
<th>Strains</th>
<th>Sequence of the signal-peptide coding region</th>
<th>Number of CT repeats</th>
<th>Gene status</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI2060</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>5</td>
<td>Off</td>
</tr>
<tr>
<td>GI2826</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>6</td>
<td>On</td>
</tr>
<tr>
<td>GI2895</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>7</td>
<td>Off</td>
</tr>
<tr>
<td>GI2924</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>9</td>
<td>On</td>
</tr>
<tr>
<td>JK44</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>3+1</td>
<td>On</td>
</tr>
<tr>
<td>JK2-41</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>3+1</td>
<td>On</td>
</tr>
<tr>
<td>JK51</td>
<td>ATGAAAAACCCCTCTTATCACTAATCTCCCTGTCTC</td>
<td>none</td>
<td>On</td>
</tr>
</tbody>
</table>

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. 3B) 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. 3A). 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. 3A) 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. 2B). 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 1 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, 34,000) and HP0796 (M, 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, 33,000 to 35,000 Ag (10). However, all of the Japanese strains had a functional oipA gene, suggesting that the M, 33,000 to 35,000 Ag noted in our previous study may be different from the oipA gene.

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