Helicobacter pylori causes gastritis, peptic ulcer, and intestinal metaplasia, and lifelong infection with this pathogen is a risk factor for gastric carcinoma and mucosal-associated B-cell lymphoma (6, 10, 14). Although the underlying mechanisms of H. pylori-associated gastroduodenal diseases are still obscure, eradication of colonizing H. pylori with antimicrobial agents markedly reduces clinical symptoms (9). Manifestations and outcomes of the diseases are dependent on a variety of factors, such as bacterial pathogenicity (8, 20), host physiology, and innate immunity (3). Escape from the host immune system is beneficial for bacterial colonization and propagation.

Recently, Tomb et al. (19) and Peck et al. (17) postulated that slipped-strand mispairing and recombination events in the H. pylori genome may evoke chromosomal variation, and those antigenically distinct proteins. We postulate that the corresponding nucleotide fragment in all 150 tested H. pylori clinical isolates by PCR or Southern blotting. The amplified Omp29-corresponding fragments were categorized into a ca. 770-bp-long group and a larger-fragment group. Sequence analysis indicated that the larger fragments were likely synthesized from the 770-bp fragments by insertion of an irrelevant fragment via 17-bp-long repeat sequences. Immunoblot analysis implies that the ca. 770-bp fragment is responsible for the protein homologous to Omp29, whereas the larger fragments are not responsible for those proteins or encoding antigenically distinct proteins. We postulate that the H. pylori outer membrane protein Omp29 can alter its antigenicity through gene modifications mediated by nucleotide transfer.

We purified a 29-kDa Helicobacter pylori outer membrane protein (Omp29 protein) and cloned the gene encoding the protein from H. pylori strain ATCC 43504. The Omp29 gene corresponded to the reported JHP73 and the HP78–79 genes of H. pylori strains. A corresponding nucleotide fragment was detected in all 150 tested H. pylori clinical isolates by PCR or Southern blotting. The amplified Omp29-corresponding fragments were categorized into a ca. 770-bp-long group and a larger-fragment group. Sequence analysis indicated that the larger fragments were likely synthesized from the 770-bp fragments by insertion of an irrelevant fragment via 17-bp-long repeat sequences. Immunoblot analysis implies that the ca. 770-bp fragment is responsible for the protein homologous to Omp29, whereas the larger fragments are not responsible for those proteins or encoding antigenically distinct proteins. We postulate that the H. pylori outer membrane protein Omp29 can alter its antigenicity through gene modifications mediated by nucleotide transfer.

**TABLE 1. Primers used for amplification and sequencing of omp29 and corresponding molecules**

<table>
<thead>
<tr>
<th>Type</th>
<th>Primer</th>
<th>Sequence (5′-3′)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification</td>
<td>jhp73S</td>
<td>CTC gCA TAT gAA AAA gAT TTT TTT AAg TAT g9C AT</td>
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<tr>
<td></td>
<td>jhp73AS</td>
<td>AAC TgC Aag Aag TTg ATC ATg TAa</td>
</tr>
<tr>
<td></td>
<td>78UPS</td>
<td>AgA AAT TCT TAg gAT TTC TCA C</td>
</tr>
<tr>
<td></td>
<td>77-3</td>
<td>CTC TgT CgA gCA TTA TAG ACT A</td>
</tr>
<tr>
<td>Sequencing</td>
<td>Ins1S</td>
<td>TTT TAg g9a gCA Tga Acg CT</td>
</tr>
<tr>
<td></td>
<td>Ins1AS</td>
<td>CAT TAA AAG CCA CgC CAT AA</td>
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<tr>
<td></td>
<td>Ins3S</td>
<td>CAg CgC TTT AAA gCg Ctg AA</td>
</tr>
<tr>
<td></td>
<td>Ins5AS</td>
<td>gCg TCT TTT TgA gCC AAT AgA</td>
</tr>
<tr>
<td></td>
<td>Ins5AS</td>
<td>AgT TCT gTg gCA Acg CgA CTA A</td>
</tr>
</tbody>
</table>

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* Lowercase “g’s” were used to avoid confusion with capital “C’s.”
FIG. 1. (A) SDS-PAGE profile of the column-purified Omp29 protein. The 29-kDa-molecular-mass Omp29 was eluted at lanes 2 to 4. (B) Immunoblotting of the SDS-PAGE gel shown in panel A, using the protein A-Sepharose-purified IgG obtained from a patient with *H. pylori* infection (1:5,000 diluted). Lanes 2 to 4 specifically reacted with the serum showing a 29-kDa-sized band. Sizes are indicated in kilodaltons. M, molecular mass markers; L, the loaded Omp29 crude sample; Ft, flowthrough fraction of the loaded Omp29 sample.

FIG. 2. Diagram of the sites of the primers (arrows) used in the study in relation to ORFs (open boxes) of each gene (thick lines). Black boxes represent the site of coding sequence for the first 16 N-terminal amino acids (omp29) and corresponding sites (*JHP73* and *HP78–79*). Hatched boxes represent the sites of 17-bp-long repeat sequence (see details in the text).
Cloning the gene encoding Omp29 protein and PCR conditions. PCR was used to amplify the gene encoding Omp29 protein from the ATCC 43504 genomic DNA. For this purpose, 100 ng of the template, 40 pmol each of jhp73S and jhp73AS primers (Table 1), and 1.25 U of Taq DNA polymerase (TaKaRa Shuzo, Kyoto, Japan) were mixed to make a 50-μl reaction mixture. PCR was performed at 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min. Genomic DNAs were extracted from H. pylori strains by the SDS-proteinase K method using CTAB (cetyltrimethylammonium bromide) (22). PCR was used with primer pairs jhp73S and jhp73AS and 78UPS and 77-3 (Table 1) under the same conditions to detect the nucleotide fragment corresponding to the Omp29 gene.

Expression of recombinant Omp29 protein and antiserum preparation. The amplified Omp29 gene was cloned into the pET21a expression vector (Novagen, Madison, Wis.) to obtain pET21a/Omp29. Escherichia coli BL21 (Stratagene, La Jolla, Calif.) was transformed with the pET21a/Omp29 plasmid and cultured in 20 ml of Luria-Bertani medium containing 100 μg/ml ampicillin. Recombinant Omp29 protein was purified by binding to an NTA agarose affinity column (Qiagen, Valencia, Calif.) and eluted with a 200-ml Ni-NTA buffer (Qiagen) containing 250 mM imidazole. The purified protein was dialyzed against 25 mM Tris-Cl (pH 7.5) and 100 mM NaCl and then stored at -80°C.

**FIG. 3.** (A) Structural analysis of omp29 and corresponding molecules. DNA sequence analyses of omp29 and corresponding molecules from SS1 and eight H. pylori clinical isolates (OMU116, OMU131, OMU73, OMU14, OMU125, OMU142, OMU136, and OMU143) were performed with a batch of primers listed in Table 1. The sequences of JHP73 of J99 and HP78–79 of 26695 were obtained from GenBank (accession numbers AE001446 and AE000529, respectively). (B) Alignment of ORF structures deduced from the DNA sequences shown in panel A. All sequences were compared and analyzed by the Vector NTI Suite version 5.5 software and the attached AlignX (InfoMax).

**TABLE 2.** Distribution and size variation of an omp29-corresponding amplicon among H. pylori clinical isolates

<table>
<thead>
<tr>
<th>Amplicon size (bp)</th>
<th>No. of strains</th>
<th>Primers used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca. 770</td>
<td>5</td>
<td>jhp73S/jhp73AS</td>
</tr>
<tr>
<td>&gt;770</td>
<td>76</td>
<td>jhp73S/jhp73AS</td>
</tr>
<tr>
<td>None</td>
<td>69</td>
<td>78UPS/77-3*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>150</strong></td>
<td></td>
</tr>
</tbody>
</table>

* Molecules corresponding to Omp29 were detected by PCR using 78UPS and 77-3 primers or by Southern blotting.
Jolla, Calif.) was transformed with pET21a/Omp29, cultured in Luria-Bertani (LB) broth, and induced by 0.4 mM IPTG (isopropylthiogalactopyranoside). A rabbit was immunized subcutaneously with 300 μg of whole-cell lysate of the transformed bacteria emulsified in Freund’s complete adjuvant and then boosted every 2 weeks. The harvested rabbit serum was adsorbed completely with E. coli BL21 lysate.

Electrophoresis and immunoblotting. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (15). Immunoblotting was performed as described in our previous work (16). Whole-cell lysate of E. coli BL21 expressing Omp29 protein, lysates of standard strains (ATCC 43504 and SS1), and lysates of time H. pylori clinical isolates were examined for reactivities with anti-Omp29 hyperimmune rabbit serum (1:10,000 diluted). The lysate of E. coli BL21 expressing Omp29 protein was also examined for reactivities using sera from eight patients infected with H. pylori (1:5,000 diluted).

DNA sequence. Amplified DNAs were sequenced by the dideoxynucleotide chain termination method with the ABI Prism 310 genetic analyzer (PE Applied Biosystems) using the primers listed in Table 1. Software Vector NTI Suite 5.5 was used for analysis. The sequence of the omp29–JHP73 gene (765 bp) of the OMU125, OMU142, OMU136, and OMU143 and the HP78 allele was also present in 81 of 150 (54%) strains as a ca. 770- to 2,040-bp-long fragment, as determined by agarose gel analysis.

The first 16 N-terminal amino acids of the purified Omp29 protein were determined as EKSGAFLGGFQYSNL, which was identical to the deduced N-terminal amino acid sequences of JHP73 (residues 18 to 33) of H. pylori strain J99 (2) and of HP78 (residues 18 to 33) of H. pylori strain 26695 (Fig. 2) (19), suggesting that omp29 corresponded to these two genes.

Cloning omp29 and distribution of a corresponding nucleotide fragment among H. pylori clinical isolates. Omp29 was amplified as a 765-bp fragment from the ATCC 43504 genomic DNA by PCR with jhp73S and jhp73AS primers. The pair of primers was designed in reference to a sequence flanking upstream and downstream of the reported JHP73 sequence of H. pylori strain J99 (Fig. 2). Genomic DNAs were extracted from 150 H. pylori clinical isolates, and PCR with the same primers was performed in order to detect a nucleotide fragment corresponding to omp29. A nucleotide fragment was amplified in 81 of 150 (54%) strains as a ca. 770- to 2,040-bp-long fragment, as determined by agarose gel analysis.

The amplicons from clinical isolates were categorized into two groups as the ca.770-bp-long group, accounting for five strains (6.2%), and amplicons larger than 770 bp, accounting for 76 strains (93.8%) out of 81 strains (Table 2). PCR with primers 78UPS (16 nucleotides [nt] upstream of jhp73S) and 77-3 (252 nt downstream of jhp73AS) primers (Table 1, Fig. 2) was performed again using these 69 negative strains. Amplicons (ca. 2,300 bp long), which were larger by the expected 268 nt, were obtained from 34 of 69 strains (Table 2). The remaining 35 PCR-negative strains were further analyzed for the presence of an omp29-corresponding fragment by Southern blotting with 32P-labeled omp29 as the detecting probe. The probe hybridized to and detected a nucleotide fragment in all 35 genomic DNA preparations (data not shown), indicating that a fragment corresponding to omp29 was also present in those 35 PCR-negative clinical isolates (Table 2).

Structural analysis of nucleotide fragments corresponding to omp29. In the next step, we analyzed the sequence homologies of omp29 and its size homologue amplicons (ca. 770 bp long) from H. pylori SS1 and OMU116 and seven larger amplicons (larger than 770 bp) from OMU131, OMU73, OMU14, OMU125, OMU142, OMU136, and OMU143. The known JHP73 gene (765 bp) of the H. pylori strain J99 and the HP78–79 gene (2,046 bp) of the H. pylori strain 26695 were also analyzed and compared. The sequence of the omp29 was homologous to those of amplicons from SS1 and OMU116 and to that of the known JHP73 of H. pylori strain J99. These ca.770-bp-long nucleotides were highly conserved throughout the whole molecules with more than 96% homology (Fig. 3A). In the larger amplicons from OMU131, OMU73, OMU14, OMU125, OMU142, OMU136, and OMU143 and the HP78–79 of H. pylori 26695, however, the highly conserved sequences shared by the four 770-bp-long molecules were divided and displaced in the 5’ (ca. 180 bp long) and 3’ (ca. 570 bp long) ends of the molecules and each pair of which was spanned by a less conserved fragment in between. Those spanning fragments composed of comparatively conserved domains located in the 5’ (ca. 210 bp, 95% identity) and the 3’ (ca. 470 bp, 87% identity) ends, and of a poorly conserved, variably sized (96 to 561 bp long) center fragment. One copy of the 17-bp-long repeat sequence GCTC(C/A)AGCCCAAG(A/C)(A/G)AC was present at the 5’ and 3’ ends of the spanning fragment and
had the same 5' to 3' sequence on the same strand. One copy of the 17-bp-long repeat sequence was also present at 181 nt from the 5' end of ca. 770-bp-long amplicons (Fig. 3A).

Figure 3B shows the open reading frame (ORF) structures deduced from the sequence of each molecule. A single ORF was depicted from the sequence of each 770-bp-long amplicon. ORFs from the larger amplicons, however, were segmented into several smaller ORFs, making the structures diverse.

**Antigenicity of Omp29-corresponding proteins in H. pylori clinical isolates and reactivity of Omp29 protein against patient sera.** Recombinant Omp29 protein was expressed in E. coli, and the whole-cell lysate separated by SDS–13% PAGE is shown in Fig. 4A. This cell lysate was used as the antigen for anti-Omp29 hyperimmune serum preparation. In immunoblot analysis with anti-Omp29 hyperimmune serum, the homologous lysate of E. coli BL21 expressing Omp29 protein and the lysate of H. pylori ATCC 43504, the parent strain of Omp29 protein, showed a 29-kDa mobility band. These results indicated that the recombinant Omp29 protein was equivalent to the original Omp29 protein with regard to antigenicity as well as estimated molecular mass. Cell lysates of H. pylori SS1 and OMU116 from which a ca. 770-bp-long amplicon was obtained reacted specifically with anti-Omp29 hyperimmune serum and showed a 29-kDa mobility band as well (Fig. 4B, lanes 2 and 3). In contrast, lysates of seven H. pylori clinical isolates from which amplicons larger than 770 bp were obtained failed to show the corresponding 29-kDa band (Fig. 4B, lanes 4 to 10).

Immunoblotting revealed that the whole-cell lysate of E. coli BL21 expressing recombinant Omp29 protein reacted specifically with eight distinct sera of H. pylori-infected patients and demonstrated a 29-kDa mobility band in each reaction (Fig. 4C).

**DISCUSSION**

*H. pylori* is involved in various lesions, including gastritis, peptic ulcer, gastric carcinoma, and mucosal-associated B-cell lymphoma. The underlying mechanisms of such pathological conditions have not yet been clarified, although several factors, such as urease (13), the Cag pathogenicity island (5, 11), VacA (4, 7), IceA (21), and BabA (12), are thought to be involved. In addition to these pathogenic factors, *H. pylori* colonize the gastric mucosa by evading the host immune system for several decades, which probably results in aggravation of the infection status. Specific host immune responses elicited in infected individuals could hardly eradicate the colonizing *H. pylori* without intervention by antimicrobial agents.

Tomb et al. (19) and Peck et al. (17) reported recently that a frameshift caused by a slipped-strand mispairing and recombinant of ATCC 43504, the parent strain of Omp29 protein; lane 2, cell lysate of SS1; lane 3, cell lysate of OMU116; lane 4, cell lysate of OMU131; lane 5, cell lysate of OMU73; lane 6, cell lysate of OMU14; lane 7, cell lysate of OMU123; lane 8, cell lysate of OMU142; lane 9, cell lysate of OMU136; lane 10, cell lysate of OMU143. (C) Immunoblotting of SDS-PAGE gel separating cell lysate of E. coli BL21 expressing Omp29 protein using eight distinct sera from patients with *H. pylori* infection (1:5,000 diluted). Each number represents serum from a patient from whom the *H. pylori* clinical strain of the same number in panel B was isolated. M, molecular mass markers.
bination events changed the antigenicity of H. pylori. This mechanism may play an important role in the escape of the bacteria from the host immunity through an antigen modification and contribute to gaining the opportunity to colonize in the stomach.

In the present study, we purified H. pylori outer membrane protein Omp29 from H. pylori type strain ATCC 43504. Our results showed that the gene encoding the protein was homologous to the known JHP73 gene by the N-terminal amino acid sequence. The omp29 was cloned from ATCC 43504 by PCR, and its size (765 bp long) and nucleotide sequence were homologous to those of JHP73 (765 bp), as predicted. In the case of H. pylori 26695, another reported genomic sequence, the 5′ terminus of omp29 aligns with the 5′ terminus of HP78 (nt 6658 to 6915), while the 3′ terminus of the omp29 aligns with the 3′ terminus of HP79 (nt 6926 to 8713) with a 1,266-bp-long less-conserved fragment in between, implying that omp29 also corresponded to HP78 together with HP79. Consequently, we speculate that the gene corresponding to omp29 is heterogeneous in size and nucleotide sequence and probably in antigenicity of the encoded protein.

A nucleotide fragment corresponding to omp29 was amplified from 115 of 150 H. pylori clinical isolates with two pairs of primers (jhp73S/jhp73AS and 78UPS/77/11032) and was further detected from the remaining 35 PCR-negative strains by Southern blotting. Therefore, a gene or a nucleotide fragment corresponding to omp29 was distributed among the entire group of 150 H. pylori clinical isolates tested in our study. The detected nucleotide fragments were heterogeneous in size, as predicted (Table 2).

Sequence analysis of the amplicons and known genes demonstrated two patterns of molecular structure, with and without an insertion fragment, which seems to be based on the size of an amplicon (Fig. 3A). The ca. 770-bp-long molecules were composed solely of well-conserved sequence with 17-bp-long repeat sequence at 181 nt from the 5′ end, whereas the larger molecules were composed of the separated well-conserved sequence at the 5′ and the 3′ ends with a less-conserved insertion sequence in between which is bracketed at both ends by the 17-bp-long repeat sequence. These components imply motifs similar to those of a site-specific recombination (18). It is possible that a less conserved fragment hopped into a gene corresponding to omp29 in a mechanism similar to a site-specific recombination and consequently made the gene heterogeneous in size and in nucleotide sequence. The 17-bp-long repeat sequence probably acted as a target sequence at the place where a less-conserved fragment was inserted. Interestingly, excision of an insertion sequence together with the 17-bp repeat(s) restores the full-length ORF in OMU131, OMU73, OMU14, and 26695 strains, suggesting a possibility of reversible expression of the gene in some strains. Recently, Alm et al. (1) reported that the JHP73 protein and HP78–79 protein are orthologous pairs and differences in their size were probably due to an intragenomic recombination. They also predicted considerable size heterogeneity of the JHP73 orthologous protein among H. pylori isolates. Their prediction is consistent with our findings, and consequently we consider Omp29 to be a JHP73-orthologous protein of ATCC 43504.

We further constructed a recombinant Omp29 protein and prepared anti-Omp29 hyperimmune serum in order to study the antigenic features of the Omp29 (JHP73)-orthologous protein of this H. pylori strain. In immunoblot analysis, anti-Omp29 hyperimmune serum reacted specifically with lysates of strain from which a ca. 770-bp-long amplicon was obtained; however, it failed to react with almost all lysates of strains from which a larger than 770-bp amplicon was obtained. One possible interpretation here is that the ca. 770-bp-long amplicon encodes an Omp29 orthologous protein antigenically homologous to Omp29, whereas a larger than 770-bp amplicon has lost the potential to encode a protein or has undergone a genetic modification to encode an antigenically distinct protein. OMU73, however, appears to possess one large ORF that would be expected to encode a ca. 50-kDa protein which would cross-react with the Omp29 protein. In the immunoblotting with anti-Omp29 serum, the OMU73 cell lysate yielded a very faint band at the position equivalent to ca. 50 kDa, suggesting the presence of the protein. The ca. 770-bp-long molecules as well as omp29 might act as a basic structure at which gene modification, probably through a fragment transfer, begins. In the present study, more than 93.8% (76 of 81) of H. pylori clinical isolates yielded larger omp29-orthologous amplicons, suggesting that these strains colonizing the stomach have undergone genetic modification, probably through a fragment transfer mechanism driven by the host immune pressure.

Intriguingly, the recombinant Omp29 protein expressed in E. coli was recognized by each of eight sera from patients with H. pylori infection tested in our study (Fig. 4C), indicating that the Omp29-homologous protein had been expressed in a colonizing H. pylori strain and exposed to the host immune system. It could be interpreted that a colonizing H. pylori strain once expressed a protein homologous to Omp29 had changed its antigenicity into a distinct one during the course of colonization. The size of an omp29-corresponding amplicon or its expression status seems not to associate directly with the clinical diagnosis of 150 patients examined.

H. pylori colonizes the gastric mucosa and subsequently proceeds to persistent infection over decades, evading the host innate immune system. One possible strategy for H. pylori to escape from the host defense mechanisms is to change the antigenicity of a protein important for immunogenicity. In addition to the presence of large paralogous outer membrane protein families (1), the Omp29 protein is a good candidate protein to be altered.

ACKNOWLEDGMENTS

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REFERENCES


