Helicobacter pylori Intrafamilial Infections: Change in Source of Infection of a Child from Father to Mother after Eradication Therapy

IKUE TANEIKE,1 YUKIKO TAMURA,1 TOSHIKI SHIMIZU,2 YUICHIRO YAMASHIRO,2 AND TATSUO YAMAMOTO1*

Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University School of Medical and Dental Sciences, Asahimachidori, Niigata,1 and Department of Pediatrics, School of Medicine, Juntendo University, Tokyo,2 Japan

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Biopsy specimens of the antrum and corpus were obtained from four Helicobacter pylori-infected members of a family and from the same boy (son 1) in whom the infection reappeared after simultaneous successful eradication treatment of three family members, excluding the mother. A total of 18 to 60 H. pylori isolates were obtained from each specimen and subjected to rRNA gene restriction pattern analysis. The father’s isolates and the initial isolates from son 1 showed the same HindIII type, which was divided into three HaeIII subtypes. Isolates from the mother and a brother (son 2) and posttreatment isolates from son 1 showed a distinct HindIII type (with one minor subtype), which was divided into six HaeIII subtypes. All subtypes of the initial isolates from son 1 were present in the father’s isolates, and all subtypes of the posttreatment isolates from son 1 were present in the mother’s isolates but not in son 2’s. Electron microscopic analysis of the biopsy specimens demonstrated extremely high levels of H. pylori colonization in the father’s gastric mucosa. H. pylori adherence with a ruffle formation was also demonstrated. The findings suggest that son 1 was infected initially with the H. pylori strain of the father and son 2 was infected with the H. pylori strain of the mother and that after eradication therapy son 1 was reinfected with the Helicobacter pylori strain of the mother, who did not undergo eradication therapy.

Helicobacter pylori colonizes the gastric mucosa via an oral route, and around 60% of individuals worldwide are infected with H. pylori (5). Children are at high risk for H. pylori infections (21, 34). H. pylori infection may persist for years or be lifelong (30, 35), although spontaneous clearance is also common in childhood (15, 19). H. pylori infection is closely associated with gastritis and peptic ulcers (14, 25), and it is also a bacterial risk factor for gastric cancer (16) and mucosa-associated lymphoid tissue lymphoma (2).

The precise mechanisms of H. pylori transmission are not yet known. Previous investigations by Drumm et al. in 1990 (8), Malaty et al. in 1991 (18), and Oderda et al. in 1991 (27) suggested intrafamilial clustering of H. pylori infections. Molecular DNA analyses of familial strains of H. pylori were then performed by Nwokolo et al. in 1992 (26) and by Bamford et al. in 1993 (1), demonstrating intrafamilial infections due to a single H. pylori strain (or a common source of infection within the family). Now, transmission among family members is considered to constitute the main route of H. pylori infection (6).

In intrafamilial H. pylori infection, the infected parents, particularly infected mothers, have been considered likely to play a key role in the transmission of H. pylori (4, 28). In contrast, in developing countries, environmental factors may be more important than intrafamilial transmission (29, 32).

Those epidemiological studies, however, are not based on molecular biological analysis of H. pylori strains. In this study, we investigated the molecular basis of H. pylori transmission by rRNA gene restriction pattern analysis (ribotyping) for the four members of a Japanese family, including one member (son 1) who showed a recurrence of infection after simultaneous, successful eradication treatment of three family members, excluding the mother. We also characterized the ultrastructure of H. pylori colonization in biopsy specimens.

MATERIALS AND METHODS

Gastric biopsy specimens. Biopsy specimens were taken from the antrum and corpus of the four H. pylori-infected members of one family at Juntendo University Hospital (Tokyo, Japan) in 1998. They included a 9-year-old boy (son 1), his 7-year-old brother (son 2), his 36-year-old father, and his 36-year-old mother (Fig. 1).

Son 1 was admitted to the hospital because of upper abdominal pain. He was positive by a [13C]urea breath test and was endoscopically diagnosed with a duodenal ulcer. The father, mother, and son 2 were also positive by a [13C]urea breath test, while the 4-year-old brother (son 3) was negative. The father and son 2 were endoscopically diagnosed with duodenal ulcers, and the mother displayed a normal gastric mucosa.

Son 1, son 2, and the father were simultaneously treated with a combination of anti-H. pylori agents (clarithromycin and amoxicillin) and a proton pump inhibitor (lansoprazole). Seven weeks after treatment, all three had negative results by [13C]urea breath tests and by histologic and culture examinations. However, 36 weeks after treatment, recurrence of the infection was observed only in son 1 by a [13C]urea breath test, and biopsy specimens were again taken from the antrum and corpus of son 1.

Media and bacterial growth. H. pylori was isolated from biopsy specimens by culturing at 37°C in a microaerophilic atmosphere (10% O2 and 10% CO2) on 5% sheep blood agar (Becton Dickinson, Tokyo, Japan) or on H. pylori-selective plates containing trimethoprim, polymyxin B, vancomycin, and amphotericin B to inhibit the growth of microbes other than H. pylori (Nissui Pharmaceuticals,
Tokyo, Japan). Single colonies were subcultured on 5% sheep blood agar and then stored at −80°C in 5% skim milk (Difco Laboratories, Detroit, Mich.) supplemented with 5% glucose (Difco). For liquid culture, H. pylori colonies on 5% sheep blood agar were suspended in brain heart infusion broth (Difco) containing 10% fetal bovine serum (Gibco, Gaithersburg, Md.), followed by incubation for 18 h at 37°C in a microaerophilic atmosphere.

**Scanning electron microscopy.** Biopsy specimens were washed in saline, fixed with 2.5% (vol/vol) glutaraldehyde in phosphate-buffered saline (pH 7.4) for 2 h at room temperature, and subsequently postfixed in 1% (wt/vol) osmium tetroxide for 1 h at 4°C. The samples were then dehydrated in acetone, critical-point dried, and coated with gold-palladium. The samples were finally analyzed by scanning electron microscopy (38, 39).

**Transmission electron microscopy.** The fixed samples were dehydrated in acetone and embedded in EPOK 812 (Oukenn Inc., Tokyo, Japan). The embedded block was cut with an ultramicrotome (MT-5000) diamond knife and stained with uranyl acetate and lead citrate. The stained samples were analyzed by transmission electron microscopy (38).

**Bacterial DNA isolation.** H. pylori DNA was prepared essentially as previously described (23). H. pylori cells grown in 10 ml of brain heart infusion broth containing 10% fetal bovine serum were suspended in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA and then lysed by the addition of sodium dodecyl sulfate (0.5%) and proteinase K (100 μg/ml). After treatment with cetrimonium bromide (1% in the presence of 0.7 M NaCl) and subsequently with chloroform-isooamyl alcohol (24:1) and phenol-chloroform-isooamyl alcohol (25:24:1), DNA in the aqueous solution was precipitated with 0.6 volume of isopropanol. DNA was then rinsed with 70% ethanol and resuspended in 50 μl of 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA.

**rRNA gene restriction pattern analysis (ribotyping).** Bacterial DNA was digested with HindIII or HaeIII, and the digests were electrophoresed in a 0.8% agarose gel with a 1-kb DNA ladder (Life Technologies, Gaithersburg, Md.) which was used as the molecular size standard. Digoxigenin (DIG)-labeled cDNA probes were prepared by reverse transcription of 16S and 23S rRNA (Roche Diagnostics GmbH, Mannheim, Germany) using a DIG DNA labeling mixture (Roche) and a cDNA synthesis kit (Roche) (36). Southern hybridization was performed as described previously (9), using a nylon membrane (Amersham International, Amersham, United Kingdom). DNA hybrid on the membrane were treated with anti-DIG alkaline phosphatase-conjugated Fab fragments (Roche) and then visualized with color development by adding nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate 4-toluidine salt (substrate) (Roche).

**Computer analysis.** Computer-assisted analysis of the ribotype patterns was performed by using a program called Molecular Analyst Fingerprinting Plus (Bio-Rad, Tokyo, Japan) according to the UPGMA clustering algorithm (24, 33). In this analysis, the Dice coefficient \[d_{AB} = 2n_{AB} / (n_A + n_B), \] where \(n_A\) is the number of bands in lane A, \(n_B\) is the number of bands in lane B, and \(n_{AB}\) is the number of bands found in both lanes A and B and the matching tolerance of 0.8% were employed.

**RESULTS**

**Ribotyping of H. pylori isolates from the family members.** A total of 18 to 60 single colonies of H. pylori were obtained from each primary culture of the antrum and corpus biopsy specimens of the family members (Fig. 1). Bacterial DNA was then obtained from each H. pylori isolate, digested with HindIII (a six-base cutter) or HaeIII (a four-base cutter, which will produce many more fragments), and subjected to ribotype analysis (Fig. 2).

When DNAs were digested with HindIII, most of the samples produced only three bands (Fig. 2A). All of the initial isolates from son 1 and the father’s isolates gave the same single ribotyping pattern, designated Fhin1 (Fig. 2A, lanes 1 to 4; Table 1). In contrast, most of the mother’s isolates, all of son 2’s isolates, plus all of the posttreatment isolates from son 1 gave a distinct single pattern, designated Mhin1 (Fig. 2A, lanes 5 to 10; Table 1). The mother’s minor isolates gave a related, similar pattern, designated Mhin2 (Fig. 2A, lane 12; Table 1).

When DNAs were digested with HaeIII, eight or more bands were observed (Fig. 2B). HaeIII digestion divided the Fhin1 type (found in the initial isolates from son 1 and the father’s isolates) into three subtypes, designated Fhae1, Fhae2, and Fhae3 (Fig. 2B, lanes 1 to 3). The Fhae1, Fhae2, and Fhae3 subtypes showed a high degree of similarity (more than 92%). The Fhae1 and Fhae2 subtypes were present in both the initial isolates from son 1 and the father’s isolates (Table 1; Fig. 2B, lanes 10 and 11), whereas the Fhae3 subtype was present only in the father’s isolates (Table 1).

HaeIII digestion also divided the Mhin1 type (found in the isolates from the mother and son 2) into five subtypes, designated Mhae1, Mhae2, Mhae3, and Bhae1, and Bhae2 (Fig. 2B, lanes 4 to 6, 8, and 9). HaeIII digestion of the Mhin2 type (found in the mother’s minor isolates) gave a distinct, sixth subtype, designated Mhae4 (Fig. 2B, lane 7). The Mhae1, Mhae2, Mhae3, and Mhae4 subtypes were close to each other, with a similarity of more than 88%. In contrast, the Fhae1 and Bhae2 subtypes were slightly distant from the Mhae1, Mhae2, Mhae3, and Mhae4 subtypes (similarity, 80 to 82%).

The mother’s isolates showed the four subtypes Mhae1, Mhae2, Mhae3, and Mhae4; of those, Mhae1 and Mhae4 were unique to the mother’s isolates (Table 1). Son 2’s isolates had the mother’s subtype, Mhae2, plus the unique subtypes Bhae1 and Bhae2 (Table 1). The posttreatment isolates from son 1’s had only two (Mhae2 and Mhae3) of the four subtypes found in the mother and no unique subtypes (Table 1; Fig. 2B, lanes 12 and 13). The Mhae2 subtype was commonly found among isolates from the mother and son 2 and posttreatment isolates from son 1.

**Characterization of H. pylori colonization by scanning electron microscopy.** Examination of son 1’s biopsy specimens showed that H. pylori had colonized the gastric mucosa of both the antrum and corpus, as shown in Fig. 3A to D. In the father’s biopsy specimens, extremely high levels of H. pylori colonization were observed, and examination revealed that the...
FIG. 2. Ribotyping analysis of *H. pylori* strains from the members of a family. *H. pylori* chromosomal DNA was digested by *HindIII* (A) and *HaeIII* (B). a, before treatment; b, after treatment. Lanes M, 1-kb DNA ladder. In panel B, *H. pylori* was from the antrum of the father (lanes 1 to 3), the mother (lanes 4 to 7), son 2 (lanes 8 and 9), and son 1 (lanes 10 to 13). The same ribotypes were also observed in *H. pylori* from the corpus.

**TABLE 1. Ribotyping patterns of *H. pylori* isolates from family members**

| Family member | Source of biopsy specimen (no. of isolates) | % of ribotyping patterns belonging to subtype<sup>a</sup> |
|---------------|-------------------------------------------|-----------------------------------------------------|
| Son 1<sup>b</sup> | Antrum (18)                              | Mhin1: 100 % Mhin2: — % Fhac1: 39 % Fhac2: 61 % Fhac3: 0 % Mhae1: — % Mhae2: — % Mhae3: — % Mhae4: — % Bhac1: — % Bhac2: — % |
|                | Corpus (24)                               | Mhin1: 100 % Mhin2: — % Fhac1: 79 % Fhac2: 21 % Fhac3: 0 % Mhae1: — % Mhae2: — % Mhae3: — % Mhae4: — % Bhac1: — % Bhac2: — % |
| Father         | Antrum (24)                               | Mhin1: 100 % Mhin2: — % Fhac1: 21 % Fhac2: 58 % Fhac3: 21 % Mhae1: — % Mhae2: — % Mhae3: — % Mhae4: — % Bhac1: — % Bhac2: — % |
|                | Corpus (24)                               | Mhin1: 100 % Mhin2: — % Fhac1: 21 % Fhac2: 42 % Fhac3: 38 % Mhae1: — % Mhae2: — % Mhae3: — % Mhae4: — % Bhac1: — % Bhac2: — % |
| Mother         | Antrum (26)                               | Mhin1: — % Mhin2: 96 % Fhac1: 4 % Fhac2: — % Fhac3: — % Mhae1: — % Mhae2: 15 % Mhae3: 65 % Mhae4: 4 % Bhac1: 0 % Bhac2: 0 % |
|                | Corpus (24)                               | Mhin1: — % Mhin2: 96 % Fhac1: 4 % Fhac2: — % Fhac3: — % Mhae1: — % Mhae2: 33 % Mhae3: 17 % Mhae4: 46 % Bhac1: 4 % Bhac2: 0 % |
| Son 2          | Antrum (28)                               | Mhin1: — % Mhin2: 100 % Fhac1: 0 % Fhac2: — % Fhac3: — % Mhae1: — % Mhae2: 0 % Mhae3: 0 % Mhae4: 14 % Bhac1: 36 % Bhac2: 0 % |
|                | Corpus (26)                               | Mhin1: — % Mhin2: 100 % Fhac1: 0 % Fhac2: — % Fhac3: — % Mhae1: — % Mhae2: 0 % Mhae3: 0 % Mhae4: 0 % Bhac1: 19 % Bhac2: 12 % |
| Son 1<sup>d</sup> | Antrum (60)                               | Mhin1: — % Mhin2: 100 % Fhac1: 0 % Fhac2: — % Fhac3: — % Mhae1: — % Mhae2: 0 % Mhae3: 0 % Mhae4: 0 % Bhac1: 0 % Bhac2: 0 % |
|                | Corpus (60)                               | Mhin1: — % Mhin2: 100 % Fhac1: 0 % Fhac2: — % Fhac3: — % Mhae1: — % Mhae2: 0 % Mhae3: 0 % Mhae4: 0 % Bhac1: 0 % Bhac2: 0 % |

<sup>a</sup> Subtypes containing “hin” were the result of *HindIII* digestion. Subtypes containing “hae” were the result of *HaeIII* digestion.

<sup>b</sup> Before eradication treatment.

<sup>c</sup> —, not detected.

<sup>d</sup> After eradication treatment.
gastric epithelium was almost completely covered by the adherent *H. pylori* (Fig. 3E). In the mother's gastric mucosa, *H. pylori* colonization was observed to a lesser extent (Fig. 3F), than in the father's specimens. Although son 1's mucosa had no detectable adherent *H. pylori* 7 weeks after eradication treatment (as expected from the negative results in culture examinations), 36 weeks after treatment *H. pylori* colonization was observed at a level similar to or even greater than that before treatment (Fig. 3G).

Unique features of *H. pylori* adherence to the mucosa. Transmission electron microscopic analysis of the biopsy specimens from son 1 before and after treatment showed that the *H. pylori*...
FIG. 3—Continued.
FIG. 4. Transmission electron micrographs showing *H. pylori* adherence to epithelial cells in gastric biopsy specimens (antrum) from son 1 before treatment (A and B) and after reinfection (C) and from the father (D). Arrowheads in panels A, C, and D point to filament-like structures between the adherent bacterium and the epithelial cells. An arrowhead in panel B points to a pedestal-like structure. Bars = 1 μm.
cells adhered to the gastric epithelial cells via filament-like structures in most cases (Fig. 4A and C). The *H. pylori* cells also occasionally bound to a pedestal-like structure (Fig. 4B). *H. pylori* adherence via filament-like structures was also observed in all other biopsy specimens examined, as shown for the father’s mucosa (Fig. 4D).

An intimate adherence characterized by ruffle formation was found in a sample from the father’s mucosa by scanning electron microscopy (Fig. 5).

**DISCUSSION**

Previous investigations using serological or histological diagnosis and/or the $[^{13}C]$urea breath test have demonstrated an intrafamilial clustering of *H. pylori* infections in Canada (8), the United States (18), Italy (7, 27), and England (1, 26). It has also been suggested that *H. pylori* is transmitted from infected parents, especially infected mothers, to children in Germany (4, 28, 29), the United States (10), and Japan (20, 22). Transmission among siblings has been suggested in Colombia (13) and Japan (22). Previous studies in England using DNA fingerprinting demonstrated a familial infection of a mother, father, and child due to a single *H. pylori* strain (1).

In this study, we carefully analyzed a case of intrafamilial infection in a Japanese family. We examined 18 to 60 *H. pylori* isolates in each biopsy specimen. In addition, we used two restriction enzymes (HindIII and HaeIII) in ribotype analysis. HindIII is a six-base cutter and HaeIII is a four-base cutter. Thus, HaeIII will produce many more fragments and ultimately has more power to subtype. For example, when examining the father’s isolates, HindIII digestion produced only one ribotype, while HaeIII digestion produced three.

In this family, before treatment the 9-year-old boy (son 1) and the father were infected with the same *H. pylori* strain and the 7-year-old boy (son 2) and the mother were infected with a different *H. pylori* strain. The 4-year-old boy (son 3) was not infected with *H. pylori*.

Son 1, the father, and son 2, who experienced duodenal ulcers, were subjected to simultaneous eradication therapy.
resulting in a successful cure 7 weeks after treatment. Thirty-six weeks later, however, son 1 was reinfected with an *H. pylori* strain with the same ribotyping pattern as one of the mother’s isolates. The mother had not been included in the eradication therapy because she displayed a normal gastric mucosa. Since the mother was the only family member who was *H. pylori* positive during son 1’s reinfection, this provides the first direct evidence of mother-to-child transmission within a family. The possibility that other environmental sources are involved in reinfection exists.

Kuipers et al. have shown that genetic drift occurs within *H. pylori* populations over the course of years of colonization within a single host (17). Indeed, analysis of *H. pylori* by Hae III digestion demonstrated variants (subtypes) with genomic mutation. All of the *H. pylori* subtypes found in son 1’s stomach before treatment were also present in the father’s stomach, and all of the *H. pylori* subtypes found in son 1’s stomach after reinfection were present in the mother’s stomach but not in son 2’s. The father, mother, and son 2 had unique *H. pylori* subtypes, whereas son 1 did not. These findings are consistent with the suggestion that, before treatment son 1 had been infected with the father’s *H. pylori* strain and son 2 had been infected with the mother’s *H. pylori* strain; after eradication therapy, son 1 became infected with the mother’s strain. The last mother-to-child transmission must have occurred within a period as short as 29 weeks.

It has been reported that younger children are more susceptible to the acquisition of *H. pylori* (13). Why only son 1 became reinfected with *H. pylori* after eradication therapy and son 2 and son 3 did not is not known. However, there is a possibility that in this family, the mother spent much more time with son 1, who was ill, and spent less time with son 2 and son 3, and thus, *H. pylori* was transmitted from the mother to son 1 (who had more contact with the mother) and not to the other two children. In addition, many older children have their own rooms in Japan, and this may also decrease the possibility of transmission among children in families.

Reinfection by *H. pylori* in adults as well as children is generally uncommon (3, 12, 31, 37). This report describes only a single family case; however, such mother-to-child transmission after treatment may be significant in Japan.

In this study, we examined biopsy specimens by electron microscopy as well as histologically. This provided the first ultrastructural findings of intrafamilial *H. pylori* infection. The father, who was the source of the initial *H. pylori* infection in the boy, had extremely high levels of *H. pylori* colonization. In the case of another familial infection in which father-to-child transmission occurred, the father again had extremely high levels of *H. pylori* colonization (unpublished data). In Japan, some fathers may have extremely high levels of *H. pylori* colonization (probably due to genetic backgrounds or eating habits that may facilitate *H. pylori* colonization in the stomach by providing cell surface receptors or bacterial growth factors) and may have a high risk of transmitting *H. pylori* to children within their families.

Thus, factors involved in the parent-to-child transmission of *H. pylori* in Japan could be the high level of *H. pylori* colonization in the stomach (e.g., the father) and frequent, close contact with the child (e.g., the mother).

Scanning electron microscopic analysis of the biopsy specimens showed tight *H. pylori* adherence to the gastric epithelial cell surface. Transmission electron microscopic analysis of the same biopsy specimens, however, showed adherence via filament-like structures in most cases. *H. pylori* may adhere to the epithelium via a surface layer such as a glycocalix or via pili. At a later stage, *H. pylori* may adhere intimately to the epithelium (6). Interestingly, in this study, ruffle formation (which resembled that of *Shigella*) (11) was observed by scanning electron microscopy. Further studies are necessary to gain insight into the precise mechanisms of *H. pylori* adherence to the human gastric mucosa as well as the circulation of *H. pylori* within a family.

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