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

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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 H. 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 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, Ichibanchou, Asahimachidori, Niigata, Japan. Phone: 81-25-227-2050. Fax: 81-25-227-0762. E-mail: tatsuoy@med.niigata-u.ac.jp.

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 (antrum and corpus) that were examined by ribotyping.

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.

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FIG. 2. Ribotyping analysis of *H. pylori* strains from the members of a family. *H. pylori* chromosomal DNA was digested by *Hind*III (A) and *Hae*III (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>
<thead>
<tr>
<th>Family member</th>
<th>Source of biopsy specimen (no. of isolates)</th>
<th>% of ribotyping patterns belonging to subtype&lt;sup&gt;a&lt;/sup&gt;:</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Corpus (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>Antrum (24)</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Corpus (24)</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Mother</td>
<td>Antrum (26)</td>
<td>—</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Corpus (24)</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>Son 2</td>
<td>Antrum (28)</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Corpus (26)</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Son 1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Antrum (60)</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Corpus (60)</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Subtypes containing “hin” were the result of *Hind*III digestion. Subtypes containing “hae” were the result of *Hae*III 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). *H. pylori* 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 reinfeeted with an \textit{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 \textit{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 \textit{H. pylori} populations over the course of years of colonization within a single host (17). Indeed, analysis of \textit{H. pylori} by HaeIII digestion demonstrated variants (subtypes) with genomic mutation. All of the \textit{H. pylori} subtypes found in son 1’s stomach before treatment were also present in the father’s stomach, and all of the \textit{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 \textit{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 \textit{H. pylori} strain and son 2 had been infected with the mother’s \textit{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 \textit{H. pylori} (13). Why only son 1 became reinfected with \textit{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, \textit{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 \textit{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 \textit{H. pylori} infection. The father, who was the source of the initial \textit{H. pylori} infection in the boy, had extremely high levels of \textit{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 \textit{H. pylori} colonization (unpublished data). In Japan, some fathers may have extremely high levels of \textit{H. pylori} colonization (probably due to genetic backgrounds or eating habits that may facilitate \textit{H. pylori} colonization in the stomach by providing cell surface receptors or bacterial growth factors) and may have a high risk of transmitting \textit{H. pylori} to children within their families.

Thus, factors involved in the parent-to-child transmission of \textit{H. pylori} in Japan could be the high level of \textit{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 \textit{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. \textit{H. pylori} may adhere to the epithelium via a surface layer such as a glycoconalix or via pilil. At a later stage, \textit{H. pylori} may adhere intimately to the epithelium (6). Interestingly, in this study, ruffle formation (which resembled that of \textit{Shigella}) (11) was observed by scanning electron microscopy. Further studies are necessary to gain insight into the precise mechanisms of \textit{H. pylori} adherence to the human gastric mucosa as well as the circulation of \textit{H. pylori} within a family.

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REFERENCES


