Evidence from a Nine-Year Birth Cohort Study in Japan of Transmission Pathways of *Helicobacter pylori* Infection

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We examined the longitudinal changes of *Helicobacter pylori* infection within 46 families with children and 48 couples without children living in Japan. The study cohort was monitored from 1986 to 1994. *H. pylori* status was assessed by the presence of anti-*H. pylori* immunoglobulin G antibodies. At study entry, *H. pylori* prevalence in children with positive mothers was 23% versus 5% in children with negative mothers (odds ratio = 5.3; 95% confidence interval = 0.6 to 42.8). Seroconversion (rate of 1.5%/year) was evident only among children living with positive mothers and did not differ among adults living with or without children. These data strongly support the cluster phenomenon of *H. pylori* infection among families, the key role of the infected mothers in the transmission within families, and the importance of adult-child transmission and not vice versa.

*Helicobacter pylori* infection is causally related to chronic gastritis, peptic ulcer disease (4, 12), and primary gastric B-cell lymphoma (5, 9). Clustering of the organism in families (3, 7, 18) suggests person-to-person transmission, a common environmental source, and a genetic basis for differences in susceptibility to the infection (19). Childhood is thought to be the primary period of risk for *H. pylori* acquisition (20, 21, 23). However, whether the organism is transmitted between adults, between adults and children, and/or between children is still not clear. We studied the pattern of *H. pylori* seroepidemiology over a 9-year period in a cohort of Japanese families living in a typical mountain village in the district of the Nagano Prefecture, Japan. We examined the longitudinal changes of the infection within families with and without children residing in the same area.

The study was carried out in the Arahiro area, a small district of South Kiso in central Japan, which is surrounded by mountains. The current water supply system was introduced in 1959 and there is a central sewage system. Adults and children were monitored between 1986 and 1994 with repeated blood sample testing and questionnaires within the framework of a study of hepatitis C transmission (14). Blood samples were collected each year; one-third of each sample volume was stored at −80°C until the current study was begun. Subjects were eligible for this study if they had at least two serum samples in two or more successive years available. Anti-*H. pylori* “eradication therapy” was not used during the period of study.

*H. pylori* status was determined by the presence of anti-*H. pylori* immunoglobulin G (IgG) antibodies in the enzyme-linked immunoabsorbent assay (ELISA) using the GAP-IgG kit, with whole-cell (*H. pylori*) extracts (Biomerica, Newport Beach, Calif.). A standard curve was drawn by measuring the absorbance of the reference serum included in the kit, which was diluted serially from 1:2 to 1:16 with phosphate-buffered saline (pH 7.2), and the amount of anti-*H. pylori* IgG corresponding to 1:8 was expressed as an arbitrary index of 1.0. The cross-reactivity of the antibody in a patient’s sera against closely related bacteria—four strains of *Campylobacter jejuni* (7.4%), one strain of *Campylobacter ralidis* (0.2%), and one strain of *Escherichia coli* (2.4%)—was examined as described previously (1, 24). In brief, sera from 10 adults and 10 children, who had been revealed to carry anti-*H. pylori* IgG, were incubated 30 min at 37°C with the sonicated cell extracts of the bacterial strains, and the unabsorbed anti-*H. pylori* IgG was measured. A control test employing an authentic *H. pylori* strain (ATCC 43504) was run in parallel. The ELISA was validated in this population by using a receiver operating characteristic curve to determine the cutoff value (arbitrary index of 0.51). When the results were compared with those obtained by bacteriological and/or histological examinations from patients with gastritis and peptic ulcer disease, the specificity and sensitivity were 93 and 96.7%, respectively (24, 27, 29).

*H. pylori* infection was defined as a positive ELISA result. A family with children was defined as at least one parent (mother) with one or more children living in the same household. The mother/wife was chosen as the index person for each family before *H. pylori* status was identified, and she was excluded from the analysis. The objective of the analyses was to compare the seroprevalence at the entry of the study among children living with positive and negative index mothers and among adults living with positive and negative index spouses. We also examined the seroconversion and seroreversion rates over the 9-year period within families with and without children. The cohort was grouped according to age when the first blood sample was taken. The data were analyzed with the SAS program (version 5; SAS Institute, Inc., Cary, N.C.).

The cohort consisted of 46 families with children, totaling 161 participants who were monitored. There were 36 positive index mothers, with 27 spouses and 62 children, and 10 negative index mothers with 7 spouses and 19 children. Forty-eight couples with no children, including 38 positive and 10 negative index wives, were monitored for the same 9-year period. Parents living with children were between the ages of 27 and 54 years, and couples without children were between the ages of 23 and 58 years.
TABLE 1. Prevalence of H. pylori infection in children and spouses according to infection status of the mothers at entry into the study (1986) in South Kiso town, Japan

<table>
<thead>
<tr>
<th>Group</th>
<th>H. pylori-positive mothers (n = 36)</th>
<th>H. pylori-negative mothers (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total H. pylori positive</td>
<td>Total H. pylori positive</td>
</tr>
<tr>
<td></td>
<td>(n = 36)</td>
<td>(n = 36)</td>
</tr>
<tr>
<td>Children</td>
<td>62 (23)</td>
<td>19 (7)</td>
</tr>
<tr>
<td>Spouses</td>
<td>27 (85)</td>
<td>7 (23)</td>
</tr>
</tbody>
</table>

**Initial data.** The prevalence of seropositivity in children living with positive mothers was five times higher than in children living with negative mothers (23 and 5%, respectively; odds ratio = 5.3; 95% confidence interval = 2.6 to 7.8). No significant difference was observed between spouses who lived with positive or negative index mothers (58 and 100%, respectively; P = 0.2) (Table 1). There were also no significant differences in seropositivity between spouses who lived with positive or negative wives (82 and 90%, respectively; P = 0.9).

**Longitudinal changes.** Of the 48 seronegative children living with positive mothers, 4 (8%) became infected by the end of the study period, while none of the 18 seronegative children living with their negative index mothers became infected. The 4 seroconverted children were between the ages of 6 and 12 years and included 2 boys and 2 girls. Of the 14 seropositive children living with positive mothers, 12 (86%) had persistence of the antibodies and 2 seroreverted by the end of the study, giving a cumulative seroreversion rate of 14%. The annual seroconversion and seroreversion rates among children (i.e., incidence and loss rate of infection) were 1.5 and 2.5%/year, respectively, based on the assumption that these rates were equally distributed throughout the mean observation period of 5.5 (standard deviation, 2.7) years. None of the 18 seronegative children or the seropositive child who lived with negative mothers had seroconverted or seroreverted (Table 2).

Of the 14 parents who were seronegative at their initial visit, one husband (7%) became infected by the end of the follow-up study. Interestingly, the wife of the seroconverted husband was seropositive while their two children were seronegative throughout the follow-up period. One (6%) of the 18 seronegative adults living with no children became infected at the end of the study. The annual seroconversion rates among adults living with and without children were 0.9 and 0.7%/year, respectively, based on the assumption that these rates were equally distributed throughout the mean observation period for adults of 7.5 (standard deviation, 2) years (Table 3). The risk of infected adults with H. pylori remaining infected did not differ between those living with and without children, 83 and 87% (P = 0.45), respectively. The annual seroreversion rates were 2.4 and 1.7%/year among adults living with and without children, respectively (Table 3).

In the present study, important points regarding transmission pathways of H. pylori infection among families were clarified. The fact that the prevalence of H. pylori infection was four times higher among children living with positive index mothers than among children living with negative index mothers confirms the clustering among families. The study provided evidence of transmission of infection from mother to child which is supported by several observations, such as seroconversion being observed only among those children living with infected mothers. Since the prevalence of H. pylori infection among mothers is much higher than in children, it is likely that the infection is transmitted from mother to child and not vice versa. Although the pattern for risk of H. pylori infection in children did not change if the index person was the father, the association with the mother is more likely to occur among Japanese families since the mother is the primary caretaker of the child. Interestingly, the four seroconverted children either had older seronegative siblings or were the only child in the family. Although sibling transmission was reported in a study from Colombia (11), we did not demonstrate such an association in the present study. This could be explained by the difference in the family structure in different cultures. For example, it is unlikely for a Japanese family to have more than three children, while it is common to have several children in a family in developing countries.

The results revealed a 2.6%/year incidence rate among adults, a slightly higher rate than that reported for adults in developed countries (0.3 to 0.6%/year) (8, 17). It is interesting that of two seroconverted adults who had seropositive spouses, one had no children and the second had two seronegative children during the observation period. This reflects the importance of the adult-to-adult or adult-to-child but not child-to-adult pathway of transmission. The identification of the organism from parents and children as the same strain of H. pylori, using DNA fingerprinting techniques, has been reported, suggesting person-to-person transmission among family members (3).

A common environmental source cannot be excluded, since parents and children share the same environment. Laboratory studies have reported that the coccoid form of H. pylori can
survive in an aquatic environment (28). Studies reported that unclean water in Peru (15) and consumption of fresh vegetables in Chile (13) have both been associated with acquisition of the infection. However, in Japan there was a rapid change in sanitary conditions after World War II and clean public water systems were introduced in the 1950s. A previous study from Japan reported a significant difference in the seroprevalence of Helicobacter pylori infection between those older than 40 years and those younger (2). It is unlikely that common environmental factors fully explain the described association, especially since the human is the only known host reservoir for Helicobacter pylori infection (30). In addition, if a common environmental source is the pathway of transmission, we would expect to observe some clustering of seroconversions among families within the study period. However, all seroconverters, including children and adults, were from different families.

There are some limitations in our study that must be considered. The small number of transmitting adults and children limited the power to identify multiple risk factors for transmission. We are unable to fully explain the route of transmission, whether it is fecal-oral or oral-oral, due to the unavailability of information on all environmental factors and household structure. Helicobacter pylori can be found in saliva, dental plaque (10, 16), and feces (6; M. A. Leverstein van Hall, A. van der Enfe, M. van Milligan de Wit, G. N. Tytgat, and J. Dankert, Letter, Lancet 342:1419–1420, 1993), which could be the vehicle of transmission through anything contaminated by them. While PCR identifies the organism in human feces, saliva, and dental plaque (22, 26; N. P. Mapstone, D. A. Lynch, and F. A. Lewis, Letter, Lancet 431:447, 1993), it is still difficult to culture the organism from materials other than gastric tissue.

In conclusion, this study demonstrated a clear pathway of transmission from adult to child and not vice versa. The knowledge that an infected mother could be the key agent for transmitting Helicobacter pylori to her children could allow the development of strategies to prevent transmission of the infection.

REFERENCES