Helicobacter pylori-Specific Immune Responses of Children: Implications for Future Vaccination Strategy

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Helicobacter pylori plays an etiologic role in gastritis, peptic ulcer disease, gastric cancer, and mucosa-associated lymphoid tissue lymphoma (2), and acquisition of the infection seems to occur mainly in the first years of life (26).

Genetic typing has shown that H. pylori is extremely diverse as a species and possesses a number of antigens that induce a specific immune response in infected individuals (1, 10, 13, 16, 17, 27). The specific immune response is part of the natural history of the infection in children, which remains poorly characterized, and may mirror the specific interaction of the host with the bacterium (8, 12, 15, 21). This immune response may be of special importance with regard to vaccination, as it may determine the antigens considered for a vaccine. However, the few existing studies of the infection in children are based mainly on symptomatic and small clinical samples (4, 7, 14, 18, 23).

The aim of this study was to analyze the specific anti-Helicobacter pylori immunoglobulin G (IgG) antibody profile for a sample of 824 asymptomatic schoolchildren in southern Germany (mean age, 10.7 ± 0.65 years) with an H. pylori-specific IgG enzyme-linked immunosorbent assay and Western blot analysis. The prevalence of infection was 19.8% (95% confidence interval, 17.1 to 22.7%). The immunoresponses were characterized predominantly by antibodies against low-molecular-mass antigens of 14 and 29 kDa, with a significant difference between children of German and Turkish nationalities (P = 0.0012 and P < 0.0001, respectively).

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H. pylori IgG antibodies were determined by enzyme-linked immunosorbent assay (GAP; Bio-Rad Laboratories Diagnostics Group, Munich, Germany). According to the manufacturer’s instructions, levels of >20 U/ml were considered positive. This test has previously been evaluated with 310 children (compared with the histology, culture, and urease test, the sensitivity of our test was 94.9% and the specificity was 92.4%) (28). The serum samples of the IgG-positive children were further analyzed for the presence of antibodies against H. pylori-specific antigens with a commercial Western blot method (Helicobacter pylori Western blot; AID, Strasbourg, Germany) as described previously (24).

Of the 824 children included in the study, 53.3% were boys. About two-thirds, or 63.5%, of the schoolchildren were of German nationality, 12.5% were Turkish, and 12.7% were of other nationalities (for 89 of the children (10.8%), there were no data available concerning nationality). Information gathered on family characteristics showed that 10.2% of the children had no siblings, 43.3% had one, and 38.1% had two siblings; 45.5% of the fathers and 37.5% of the mothers had 9 or fewer years of school education.

One hundred sixty-three children (19.8% of the total number, 95% confidence interval [CI], 17.1 to 22.7%) had positive serology results for H. pylori (Table 1). The seroprevalence in children of German nationality was 13.9% (95% CI, 11.0 to 17.1%) and varied from 12.4% (95% CI, 9.6 to 15.6%) for the German children born in Germany to 42.3% (95% CI, 23.4 to 63.1%) for the German children born elsewhere (mostly children of families who had lived in countries of Eastern Europe for generations and who had returned to Germany quite recently). The prevalence of H. pylori in children of Turkish nationality was 37.9% (95% CI, 28.5 to 48.0%) (in Turkish children, place of birth was not associated with seroprevalence), and for children of other nationalities, it was 30.5% (95% CI, 21.9 to 40.2%). These strong differences among ethnic groups were independent of socioeconomic factors, such as family size and school education of parents, and persisted after these factors had been controlled for. On the other hand, duration of school education of parents and number of siblings were not factors associated with seroprevalence if ethnic origin was considered in the analysis (data not shown).
Western blots were used to detect and analyze eight different *H. pylori*-specific immunoreactive bands (Table 2).

Table 3 shows that antibodies to the 25-kDa antigen and the 14-kDa antigen were detected much more frequently in infected children of Turkish or other nationality than in infected children of German nationality ($P$ values of 0.0012 and <0.0001, respectively).

This study, conducted with a population-based sample of asymptomatic schoolchildren in Germany, demonstrated that the specific systemic immune response in children to *H. pylori* infection is particularly pronounced against low-molecular-weight proteins of *H. pylori*. Furthermore, there was a large variation in both the prevalence of infection and the specific antibody patterns with respect to the nationalities of the children, although the children were living in the same geographic region. As shown in a previous study, differences between ethnic groups are not explained by differences in socioeconomic factors (25) and most likely mirror strain differences among these groups.

A recent French study of 80 pediatric patients found the highest prevalence (88.7%) for antibodies to a 26-kDa antigen (22). Little is known about the function of the 26-kDa antigen, which may be identical to the 25-kDa antigen in our study. However, recently it has been shown that a 25-kDa outer membrane protein acts in a lectin-like manner with lipopolysaccharide to mediate attachment of *H. pylori* to laminin and may therefore be important for the colonization process in the early phase of the infection (11, 29).

Much interest exists in the seroprevalence in children of CagA antibodies, which, according to a study by Mitchell et al. (14), correlated with age and increased up to the age of 15 years. There are few data available for children, and the results of recent studies have been inconsistent concerning the prevalence and association with disease of CagA-positive strains in both adults (16, 19, 28) and children (3, 6, 7, 9, 12, 28, 30). This may be due partly to the considerable variation in the frequencies of vacA*St* subtypes and ica*A alleles in children originating from different geographic regions (19, 20). As for adults, a high prevalence of CagA-positive *H. pylori* strains was found in Japanese children; however, there was no association of this prevalence with nodular gastritis or peptic ulcer disease (7) in Japanese children (mean age, 12 ± 3 years). This result challenges the role of CagA as a marker for more serious gastrointestinal disease in children as well as in adults in Japan (7). The highest prevalence of CagA antibodies in Europe (80%), has been found in a study of infected children in Poland (5); the prevalence in infected schoolchildren in Estonia (9 to 15 years old) was 46% (30). Raymond et al. described the prevalence of CagA antibodies in children in France who had abdominal pain to be 43% (22).

In conclusion, the differences in the antibody patterns between children of various ethnic groups or found in our study may indicate strain differences and should be considered if antigens are chosen as targets for a vaccination approach.

**REFERENCES**

1. Aucher, P., M. L. Petit, P. R. Mannant, L. Pezenneb, P. Babin, and J. L. Faucher. 1998. Use of immunoblot assay to define serum antibody patterns associated with *Helicobacter pylori* infection and with *H. pylori*-related ulcers. J. Clin. Microbiol. 36:931–936.

2. Blaser, M. J., and D. E. Berg. 2001. *Helicobacter pylori* genetic diversity and risk of human disease. J. Clin. Investig. 107:767–773.

3. Camorlinga-Ponce, M., J. Torres, G. Perez-Perez, Y. Leal-Herrera, B. Gonzalez-Ortiz, A. Madrazo de la Garza, and O. Munoz. 1998. Validation of a serologic test for the diagnosis of *Helicobacter pylori* infection and the immune response to urease and CagA in children. Am. J. Gastroenterol. 93:1264–1270.

4. Elitsur, Y., C. Neace, M. C. Wertherhammer, and W. E. Triest. 1999. Prevalence of CagA, VacA antibodies in asymptomatic and asymptomatic children with *Helicobacter pylori* infection. *Helicobacter* 4:100–105.

5. Gylz, A., D. E. Berg, and D. Dzierzanowska. 1997. Epidemiology of vacA*St* alleles in *H. pylori* isolated from children and adults in Poland. J. Physiol. Pharmacol. 48:333–343.

6. Husson, M.-O., F. Gottrand, A. Vache, L. Dhennens, E. Martin De La Salle, D. Turck, M. Houcke, and H. Leclerc. 1995. Importance in diagnosis of gastritis of detection by PCR of the cagA gene in *Helicobacter pylori* strains isolated from children. J. Clin. Microbiol. 33:3300–3303.
7. Kato, S., T. Sugiyama, M. Kudo, K. Ohnuma, K. Ozawa, K. Inuma, M. Asaka, and M. J. Blaser. 2000. CagA antibodies in Japanese children with nodular gastritis or peptic ulcer disease. J. Clin. Microbiol. 38:68–70.
8. Kumagai, T., H. Malaty, D. Y. Graham, et al. 1998. Acquisition versus loss of Helicobacter pylori infection in Japan: results from an 8-year birth cohort study. J. Infect. Dis. 178:717–721.
9. Laheij, R. J. F., H. Straatman, J. B. M. J. Jansen, and A. L. M. Verbeek. 1998. Evaluation of commercially available Helicobacter pylori serology kits: a review. J. Clin. Microbiol. 36:2803–2809.
10. Lamarque, D., T. Gilbert, F. Roudot-Thoraval, L. Deforges, M. T. Chaumette, and J. C. Delchier. 1999. Seroprevalence of eight Helicobacter pylori antigens among 182 patients with peptic ulcer, MALT gastric lymphoma or non-ulcer dyspepsia. Higher rate of seroreactivity against CagA and 35-kDa antigens in patients with peptic ulcer originating from Europe and Africa. Eur. J. Gastroenterol. Hepatol. 11:721–726.
11. Leal-Herrera, Y., J. Torres, G. Perez-Perez, A. Gomez, and T. Monath. 1999. Serologic IgG response to urease in Helicobacter pylori infected persons from Mexico. Am. J. Trop. Med. Hyg. 60:587–592.
12. Malaty, H., D. Y. Graham, W. Wattigny, S. Srinivasan, M. Osato, and G. Berenson. 1999. Natural history of Helicobacter pylori infection in childhood: a 12-year follow-up cohort study in a biracial community. Clin. Infect. Dis. 28:279–282.
13. Mattsson, A., A. Tinnert, A. Hamlet, H. Lonroth, I. Bölin, and A.-M. Svennerholm. 1998. Specific antibodies in sera and gastric aspirates of symptomatic and asymptomatic Helicobacter pylori-infected subjects. Clin. Diagn. Lab. Immunol. 5:288–293.
14. Mitchell, H. M., S. T. Hazell, T. D. Bohane, P. Hu, M. Chen, and Y. Y. Li. 1999. The prevalence of antibody to CagA in children is not a marker for specific disease. J. Pediatr. Gastroenterol. Nutr. 28:71–75.
15. Mitchell, H. M., S. L. Hazell, T. Kolesnikow, J. Mitchell, and D. Frommer. 1996. Antigen recognition during progression from acute to chronic infection with a cagA-positive strain of Helicobacter pylori. Infect. Immun. 64:1166–1172.
16. Mitchell, H. M., S. L. Hazell, Y. Y. Li, and P. J. Hu. 1996. Serological response to specific H. pylori antigens: antibody against CagA antigen is not predictive of gastric cancer in a developing country. Am. J. Gastroenterol. 91:1785–1788.
17. Nilsson, L, A. Ljung, P. Aleljung, and T. Wadstrom. 1997. Immunoblot assay for serodiagnosis of Helicobacter pylori infections. J. Clin. Microbiol. 35:427–432.
18. Oliveira, A. M. R., G. A. Rocha, D. M. M. Queiroz, E. N. Mendes, A. S. T. Carvalho, T. A. Ferrari, and A. M. M. F. Nogueira. 1999. Evaluation of enzyme-linked immunosorbent assay for the diagnosis of Helicobacter pylori infection in children from different age groups with and without duodenal ulcer. J. Pediatr. Gastroenterol. Nutr. 28:157–161.
19. Paoluzi, O. A., P. Rossi, C. Montesano, S. Bernardi, E. Carnieri, O. P. Marchione, F. Nardi, F. Iacopini, R. Pica, and P. Paoluzi. 2001. Discrepancy between polymerase chain reaction assay and Western blot analysis in the assessment of CagA status in dyspeptic patients. Helicobacter 6:130–135.
20. Perez-Perez, G. I., N. Bhat, J. Gaensbauer, F. Alan, D. N. Taylor, E. J. Kuipers, L. Zhang, W. C. You, and M. J. Blaser. 1997. Country-specific constancy by age in cagA+ proportion of Helicobacter pylori infection. Int. J. Cancer 72:453–456.
21. Perri, F., M. Pastore, R. Clemente, V. Festa, M. Quittadamo, G. Niro, P. Conoscoitore, P. Rutgeerts, and A. Andriulli. 1998. Helicobacter pylori infection may undergo spontaneous eradication in children: a 2-year follow-up study. J. Pediatr. Gastroenterol. Nutr. 27:181–183.
22. Raymon, J., C. Sauvestre, N. Kalach, M. Bergeret, and C. Dupont. 2000. Immunoblotting and serology for diagnosis of Helicobacter pylori infection in children. Pediatr. Infect. Dis. J. 19:118–121.
23. Rocha, G. A., A. M. R. Oliveira, D. M. M. Queiroz, A. S. T. Carvalho, and A. M. M. F. Nogueira. 2000. Immunoblot analysis of humoral immune response to Helicobacter pylori in children with and without duodenal ulcer. J. Clin. Microbiol. 38:1777–1781.
24. Rokkas, T., S. Ladas, C. Liatsos, E. Petridou, G. Papatheodorou, S. Theocharis, A. Karameris, and S. Raptis. 1999. Relationship of Helicobacter pylori CagA status in gastric cell proliferation and apoptosis. Dig. Dis. Sci. 44:487–493.
25. Rothenbacher, D., G. Bode, G. Berg, R. Gommel, T. Gonser, G. Adler, and H. Brenner. 1998. Prevalence and determinants of H. pylori infection in preschool children—a population based epidemiological study from Germany. Int. J. Epidemiol. 27:135–141.
26. Rothenbacher, D., J. Incougla, G. Bode, and H. Brenner. 2000. Acquisition of Helicobacter pylori infection in a high-risk population occurs within the first 2 years of life. J. Pediatr. 136:744–748.
27. Shieh, S. C., B. S. Sheu, H. B. Yang, H. J. Tsao, and X. Z. Lin. 2000. Serologic response to lower-molecular-weight proteins of H. pylori is related to clinical outcome of H. pylori infection in Taiwan. Dig. Dis. Sci. 45:781–788.
28. Sim, J. G., E. C. Kim, and J. K. Seo. 1996. The role of serology in the diagnosis of Helicobacter pylori infection in children. Clin. Pediatr. 34:458–462.
29. Valkonen, K. H., T. Wadstrom, and A. P. Moran. 1997. Identification of the n-acetylmuraminylactosamine-specific laminin-binding protein of Helicobacter pylori. Infect. Immun. 65:916–923.
30. Vorobjova, T., H. Grunberg, M. Ouma, H. I. Maaroos, I. Nilsson, T. Wadstrom, A. Covacci, and R. Ulbo. 2002. Seropositivity to Helicobacter pylori and CagA protein in school children of different ages living in urban and rural areas in southern Estonia. Eur. J. Gastroenterol. Hepatol. 12:97–101.