

Helicobacter pylori and Specific Immunoglobulin E Antibodies to Food Allergens in Children

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ABSTRACT

Objectives: It has been suggested that chronic *Helicobacter pylori* infection may increase gastric permeability, predisposing infected children for the development of food allergies. We assessed the presence of food-specific immunoglobulin (Ig)E antibodies in *H. pylori* positive children and controls.

Methods: We measured specific IgE values to six major food allergens (Pharmacia CAP-system) in a group of school-aged Caucasian (n = 36) and non-Caucasian (n = 38) children with a known *H. pylori* status. All children had undergone upper gastrointestinal endoscopy because of abdominal complaints.

Results: Among *H. pylori* positive children (mean age, 8.8 years; range, 5–15 years, 25 female, 26 male), 33% (17 of 51) had an elevated food-specific IgE level to at least one of the food allergens tested. Unexpectedly, the majority of those with

elevated serum food-specific IgE levels (12 of 17) were to cow's milk. Among *H. pylori* negative children (mean age, 9.3 years; range, 5–15 years, 13 female, 10 male), 26% (6 of 23) of the children had an elevated serum IgE level to at least one of the food allergens tested, and 9% (2 of 23) were positive to cow's milk. The difference in the number of children with an elevated serum IgE level for cow's milk in *H. pylori* positive and negative children was not significant. The severity of gastritis did not correlate with the presence of food-specific IgEs.

Conclusions: *H. pylori* infection had no effect on the manifestation of specific IgE to major food allergens in school-aged children. An IgE response to cow's milk was common among these school-aged children. *JPGN* 40:180–183, 2005.

Key Words: childhood—food allergens—*H. pylori*. © 2005 Lippincott Williams & Wilkins

INTRODUCTION

Chronic *Helicobacter pylori* infection has an effect on gastric permeability that increases with increasing inflammatory activity (1,2). It has been suggested that this increase in permeability in the stomach may lead to increased food antigen exposure and thereby have an effect on the development of immunoglobulin (Ig)E-antibodies to food antigens. Interestingly, total IgE in serum is high in *H. pylori* infected children without any signs of other diseases that might elevate IgE levels (3).

Recently Corrado et al. (4) reported that *H. pylori* is a common finding in children with IgE-mediated food-allergy. They found increased IgG titers to *H. pylori* in 30% of their patients with food allergy whereas the respective figure was only 10% in children with asthma and 0% in those with inflammatory bowel disease. In adults, it has been suggested that the presence of more virulent *H. pylori* may increase the risk of developing

food allergy (5), but this has not been confirmed in children (4). In atopic patients, *H. pylori* infection increases the intensity of gastric inflammation (6). If there is a positive association between *H. pylori* and development of food allergy, it probably takes years to appear. In a short report on infants with food allergy, only one such child was positive for *H. pylori* (7). Therefore, we studied serum IgE antibody levels to major food allergens (egg, milk, peanut, fish, wheat, soybean; Pharmacia CAP-system) in *H. pylori* infected and noninfected school-aged children.

SUBJECTS AND METHODS

All 74 children included in this study underwent upper gastrointestinal endoscopy at the Day Surgery Unit of Hospital for Children and Adolescents, Helsinki University, during 2000–2002 because of abdominal symptoms. Endoscopies were performed because of a suspected *H. pylori* infection (n = 42) and because of abdominal pain (n = 17), gastrointestinal reflux (n = 5), exclusion of coeliac disease (n = 6), vomitus (n = 2) or melena (n = 2). Routine biopsies were taken from antrum, corpus and duodenum and the degree of gastritis was assessed according to the Sydney system (8). None of the children had peptic ulcer on endoscopy.

Serum IgG and IgA antibodies to *H. pylori* were measured by an in-house enzyme immunoassay (9). The lower limit of raised titers was 700 for IgG antibodies. With this limit, the sensitivity

Received February 17, 2004; accepted June 16, 2004.

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Supported by Helsinki University Research Funds, Helsinki University Central Hospital Grant 2303, Finnish Academy, Sigrid Jusélius Foundation.

of the assay is 93% and specificity 97% for IgG antibodies to *H. pylori*. In IgA antibodies, the lower limit for raised titers was 70, giving sensitivity of 64% and specificity of 98% (9). CagA antibodies were measured with a commercial immunoblot method (I.D. Blot *H. pylori* IgG; DPC, Los Angeles, CA) in accordance with the instructions of the manufacturer. Positive bands were estimated visually.

The group of *H. pylori* positive children (n = 51) included those with elevated titers of *H. pylori* antibodies in their sera and either a positive culture for helicobacteria (n = 46) or a positive rapid urease test (Jatrox) on endoscopy (n = 5) and helicobacteria seen on histologic examination. Their *H. pylori* negative controls (n = 23) were age- and sex-matched children who had their *H. pylori* antibodies measured and underwent upper gastrointestinal endoscopy at the same time at our hospital but were found negative for *H. pylori* (rapid urease test negative and no helicobacteria seen on histologic examination of gastric biopsy). Among these children, *H. pylori* antibody titers were below the lower limit for raised titers.

In the *H. pylori* positive group (n = 51), the mean age was 8.8 years (range, 5–15 years, 25 female, 26 male; Caucasians n = 16, non-Caucasians n = 35). In the *H. pylori* negative group (n = 23), the mean age was 9.3 years (range, 5–15 years; 13 female, 10 male; Caucasians n = 20, non-Caucasians n = 3). This *H. pylori* positive group included all consecutive *H. pylori* positive children diagnosed during 2000 to 2002 whose blood samples could be traced retrospectively. A total of 399 children >5 years of age underwent upper gastrointestinal endoscopy at our Day Surgery Unit during 2000–2002, and the routine rapid urease test was positive in 25% of these cases. Thus, it is estimated that 60% of all *H. pylori* positive children diagnosed during 2000–2002 were included in the present study.

The Phadebas radioallergosorbent test (RAST) method (Pharmacia CAP-system, (10) was used for measurement of specific IgE against major food allergens (wheat, fish, peanut, egg, soybean, cow's milk). Values equal to or higher than 0.35 kU/L (corresponding to RAST class 1 or greater) were considered positive. The study was approved by the Ethics committee of Helsinki University Central Hospital.

Statistical Analyses

The Mann-Whitney *U*-test and Spearman's rank correlation test were used for nonparametric tests. Fisher's exact two-sided test was used for comparisons of the numbers of IgE positive children within *H. pylori* positive or negative groups. Comparisons were considered significant at *P* < 0.05.

RESULTS

For the *H. pylori* positive children, 33% of them (17 of 51) were positive to at least one of the food allergens

tested. Food-specific IgE values ranged from 0.37 kU/L to 16.1 kU/L, and 53% (9 of 17) of the IgE positive children had values greater than 0.7 kU/L (RAST class 2; Table 1). Two children had values greater than 3.5 kU/L (RAST class 3). The majority of IgE positive children (11 of 17) were positive for two or more food allergens. The specific IgE to milk was the major cause of positive reactions (Table 1).

Regarding the *H. pylori* negative children, 26% of them (6 of 23) were positive for at least one of the food allergens tested and four children were IgE positive for more than one food antigen tested (Table 1). Two children (9% of the *H. pylori* negative children) were positive to milk antigen. Specific IgE values ranged from 0.35 kU/L to 100 kU/L and three of six (50%) children had values greater than 0.7 kU/L (RAST class 2) (Table 1). Two children had values greater than 3.5 kU/L (RAST class 3). The differences between *H. pylori* positive and negative children were not significant (*P* > 0.05)

Age, sex, or season during blood sampling had no effect on the IgE positivity to food allergens. The IgE positivity to specific food allergens varied according to ethnicity (Table 2) but the differences did not reach statistical significance (*P* > 0.05). Three children had a diagnosis of atopic disease.

Serum IgG or IgA antibodies to *H. pylori* or the presence of CagA antibodies (detectable in 80% of the *H. pylori* infected children) had no significant association with IgE levels to food allergens. In *H. pylori* infected children, the degree of gastritis was higher (median grade, 2; range, 1–3) than in *H. pylori* negative children (median grade, 0; range, 0–2; *P* < 0.0001). The severity of gastritis did not correlate with the presence of food-specific IgEs. Four children had duodenitis in histologic examination. Three of these four children had positive screening results for food-specific IgEs (RAST class 1 in two children and RAST class 2 in one child).

DISCUSSION

It has been suggested that *H. pylori* may be related to the development of food sensitization (3–5) but the data on children are limited. We screened for food-specific IgE levels in 74 school-aged children who had undergone upper gastrointestinal endoscopy and thus had *H. pylori* status assessed on histologic examination of their gastric

TABLE 1. Specific IgE to food allergens (egg, milk, peanut, fish, wheat, soy-bean) in serum among *H.pylori* positive and negative school-aged children

	IgE > 0.35 kU/L to at least one food allergen	IgE > 0.35 kU/L to more than one food allergen	IgE > 0.35 kU/L to cow's milk	IgE > 0.7 kU/L to at least one food allergen
<i>H. pylori</i> positive (n = 51)	17 (33%)	11 (22%)	12 (24%)	9 (18%)
<i>H. pylori</i> negative (n = 23)	6 (26%)	3 (13%)	2 (9%)	3 (13%)
<i>P</i> value (Fisher's exact 2-sided test)	0.60	0.53	0.51	0.74

Values are expressed as n (%).
Ig = immunoglobulin.

TABLE 2. Ethnicity and specific IgE to food allergens in the serum of school-aged children

	IgE > 0.35 kU/L to at least one food allergen	IgE > 0.7 kU/L to at least one food allergen	IgE > 0.35 kU/L (RAST class 1 or higher)					
			Cow's milk	Egg white	Wheat	Peanut	Soybean	Fish
Caucasian (n = 36)	10 (28%)	4 (11%)	6 (17%)	5 (14%)	3 (8%)	3 (8%)	2 (6%)	0 (0%)
Non-Caucasian (n = 38)	13 (34%)	7 (18%)	8 (21%)	3 (8%)	6 (16%)	6 (16%)	6 (16%)	1 (3%)
P value (Fisher's exact 2-sided test)	0.62	0.52	0.77	0.47	0.48	0.48	0.26	1

Values are expressed as n (%).
Ig = immunoglobulin.

biopsies. Food-specific IgEs were common, as 30% of the children studied had detectable levels in their sera, but there was no difference between *H. pylori* positive and negative children. There was no correlation between the food-specific IgEs and the severity of *H. pylori* infection (as assessed by the degree of gastritis, levels of *H. pylori* antibodies or the presence of CagA antibodies) (9). The majority of the *H. pylori* positive children in our series were culture-positive for *H. pylori* and had elevated levels of antibodies to *H. pylori* in their sera, which suggests a long-term rather than transient colonization with *H. pylori* (9,11). Accordingly, the *H. pylori* negative group comprised children with no evidence of *H. pylori* in their stomachs. A negative finding of helicobacteria on histologic examination does not necessarily exclude a recent *H. pylori* infection, as spontaneous eradication of *H. pylori* is possible in children (12). After elimination of *H. pylori*, however, gastric inflammation is present for several weeks or even for months (13). Thus, as the *H. pylori* negative children had not more than minor inflammatory changes in their gastric biopsies and their *H. pylori* antibody titers were low, it is likely that they were true negatives and had not, for instance, had a recent chronic infection with *H. pylori*.

Specific IgE values in serum were clearly elevated (greater than 0.7 kU/L, RAST class 2) in 16% and slightly elevated (greater than 0.35 kU/L, RAST class 1) in 31% of the children studied. It is far from clear to what degree school-aged children have increased levels of specific IgE to food allergens. At the age of 10 years, 20% of Finnish children have specific IgEs to inhalant allergens in their sera whereas only 3% are IgE positive to egg white (14). Unfortunately, the study does not contain data on IgE levels against food allergens other than egg white. Egg white is, however, considered to be the most potent sensitizing food allergen (15,16). Thus, in our study the proportion of school-aged children with cow's milk IgEs was high (19%). It must be born in mind that every child in the present study had undergone upper gastrointestinal endoscopy as a result of abdominal

complaints. Therefore, it is possible that the presence of food-specific IgEs is related to the development of gastrointestinal symptoms among these children. It is important to bear in mind that the finding of food-specific IgEs is not equivalent to food allergy. As our series was a retrospective analysis, it was unfortunately impossible to perform food challenge tests.

As stated above, the food-specific IgE most often elevated was not egg white but the specific IgE to cow's milk. The antigen used in milk IgE test is a mixture of milk proteins, including casein and whey proteins (10). Beta-lactoglobulin is the major immunogenic protein in milk; it is about 36,000 D in size, and it has been used as a marker protein for gut permeability (17). It is an intriguing suggestion that increased responses to milk allergens might reflect an increase in gastrointestinal permeability either in the stomach or in the gut. In *H. pylori* infected children, however, the gastric permeability was not increased when measured with the sucrose test (18), which is another marker used for the measurement of permeability and a diagnostic tool for detecting mucosal gastric damage in children also (19). As the penetration of proteins and sugars may differ in patients with mucosal damage (20), an unaffected sucrose test does not exclude alterations in permeability of the gut or the stomach. There is some evidence that *H. pylori* modify the epithelial barrier, resulting in an increased passage of macromolecules via transcellular pathway (21). This increase in transepithelial transport is suggestive for an increase in antigenic load (22). In our series, specific IgEs to cow's milk were frequent among *H. pylori* infected children, but whether this reflects an increased absorption of milk proteins remains to be studied.

The "hygiene hypothesis" has suggested that the manifestation of allergic diseases increases when the number of early infections decreases along the development of housing condition and social manners. Kosunen et al. (23) have shown that IgE positivity for inhalant allergens increases in a population at the same time that the proportion of *H. pylori* seropositivity

decreases. This study was not designed to explore the causality, and the result may also reflect the overall improved hygienic level of living for which decrease of *H. pylori* seropositivity is a surrogate marker. There is no data on the relation of *H. pylori* seropositivity and IgE positivity for food allergens. Among Finnish children, seropositivity to *H. pylori* is uncommon (24), as the decreasing trend in seropositivity in Finnish adults also suggests (23). This is in contrast to the non-Caucasian children inhabited in Finland, as the majority of them are *H. pylori* positive (25) and they comprise the majority of *H. pylori* infected children diagnosed at our hospital. If *H. pylori* is one of the possible infections that would decrease the development of allergen-specific IgE responses, one would expect that inhalant- or food-specific IgE levels would be low in *H. pylori* positive children. In our study, however, the food-specific IgE levels were similar in the *H. pylori* positive and negative children. Although the number of children included in our series is limited, this finding may suggest that *H. pylori* positivity does not seem to protect children living in a developed society from the development of IgE responses.

In peptic ulcer disease, total IgE-level may increase as indicated by the results of Lazzari et al. (26). Peptic ulcers are rare among *H. pylori* positive children in Finland, and at our hospital only a few cases are seen each year. This is in striking contrast to a Russian study reporting high IgE levels in *H. pylori* infected children, as 20% of those children had duodenal ulcers (3). As the sample volume was limited in our study we could not measure total IgE in our children but levels were probably low, as none of the *H. pylori* positive children had peptic ulcers.

Our results showed that there was no difference in food-specific IgE levels between *H. pylori* positive and negative children. Thus, it seems that *H. pylori* plays a minor pathophysiologic role in the development of IgE responses to food allergens in children.

Acknowledgements: Mr. Peter Elg and Ms. Pirjo Kosonen are thanked for excellent technical assistance.

REFERENCES

1. Borch K, Sjostedt C, Hannestad U, et al. Asymptomatic *Helicobacter pylori* gastritis is associated with increased sucrose permeability. *Dig Dis Sci* 1998;43:749–53.
2. Fukuda Y, Bamba H, Okui M, et al. *Helicobacter pylori* infection increases mucosal permeability of the stomach and intestine. *Digestion* 2001;63 Suppl 1:93–6.
3. Studenikin MYa, Nijevitch AA, Mutalov AG. Consideration of *Helicobacter pylori* infection in childhood: immune response, endoscopic and morphological findings. *Acta Paediatr Jpn* 1995; 37:551–6.
4. Corrado G, Luzzi I, Lucarelli S, et al. Positive association between *Helicobacter pylori* infection and food allergy in children. *Scand J Gastroenterol* 1998;33:1135–9.
5. Figura N, Perrone A, Gennari C, et al. CagA-positive *Helicobacter pylori* infection may increase the risk of food allergy development. *J Physiol Pharmacol* 1999;50:827–31.
6. Bartuzi Z, Korenkiewicz J, Romanski B. Correlation between *Helicobacter pylori* infection and food allergy in chronic gastritis. *Med Sci Monit* 2000;6:530–8.
7. Roehr CC, Reibel S, Niggeman B. Children with food allergy presenting as atopic dermatitis (AD) were compared with patients with food allergy and gastrointestinal symptoms. *Pediatr Allergy Immunol* 2001;12:112.
8. Price AB. The Sydney system: histological division. *J Gastroenterol Hepatol* 1991;6:209–22.
9. Kolho K-L, Karttunen R, Heikkilä P, et al. Gastric inflammation is enhanced in children with CagA-positive *Helicobacter pylori* infection. *Pediatr Infect Dis J* 1999;18:337–41.
10. Ahlstedt S, Holmqvist I, Kober A, et al. Accuracy of specific IgE antibody assays for diagnosis of cow's milk allergy. *Ann Allergy Asthma Immunol* 2002;89(Suppl):21–5.
11. Kolho K-L, Korhonen J, Verkasalo M, et al. *Helicobacter pylori* serology at diagnosis and follow-up of biopsy-verified infection in children. *Scand J Infect Dis* 2002;34:177–82.
12. Perri F, Pastore M, Clemente R, et al. *Helicobacter pylori* infection may undergo spontaneous eradication in children: a 2-year follow-up study. *J Pediatr Gastroenterol Nutr* 1998;27:181–3.
13. Wittman EM, Mravunac M, Bexx MJC, et al. Improvement of gastric inflammation and resolution of epithelial damage one year after eradication of *Helicobacter pylori*. *J Clin Pathol* 1995;48:250–6.
14. Siltanen M, Kajosaari M, Pohjavuori M, et al. Prematurity at birth reduces the long-term risk of atopy. *J Allergy Clin Immunol* 2001; 107:229–34.
15. Hattevig G, Kjellman B, Johansson SGO, et al. Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. *Clin Allergy* 1984;14:551–9.
16. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107:891–6.
17. Kuitunen M, Savilahti E, Sarnesto A. Human α -lactalbumin and bovine β -lactoglobulin absorption in infants. *Allergy* 1994;49:354–60.
18. Gotteland M, Corvalan A, Sarmiento F, et al. Gastric permeability is not increased in children colonized by CagA-positive strains of *Helicobacter pylori*. *Dig Liver Dis* 2001;33:750–4.
19. Vera JF, Gotteland M, Chavez, et al. Sucrose permeability in children with gastric damage and *Helicobacter pylori* infection. *J Pediatr Gastroenterol Nutr* 1997;24:506–11.
20. Kuitunen M, Savilahti E. Gut permeability to human α -lactalbumin and bovine β -lactoglobulin, mannitol and lactulose in coeliac disease. *J Pediatr Gastroenterol Nutr* 1996;22:197–204.
21. Matysiak-Budnik T, Terpend K, Alain S, et al. Antigen absorption and processing in a digestive tract epithelial cell line model. *Infect Immun* 1998;66:5785–91.
22. Matysiak-Budnik T, Hashimoto K, Heyman M, et al. Antral gastric permeability to antigens in mice is altered by infection with *Helicobacter felis*. *Eur J Gastroenterol Hepatol* 1999;11:1371–7.
23. Kosunen TU, Höök-Nikanne J, Salomaa A, et al. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002;32:373–8.
24. Rehnberg-Laiho L, Rautelin H, Valle M, et al. Persisting *Helicobacter* antibodies in Finnish children and adolescents between two and twenty years of age. *Pediatr Infect Dis J* 1998;17:796–9.
25. Kolho KL, Savilahti E. Ethnic differences in intestinal disaccharidase values in children in Finland. *J Pediatr Gastroenterol Nutr* 2000;30:283–7.
26. Lazzari de F, Mancin O, Plebani M, et al. High IgE serum levels and "peptic ulcers": clinical and functional approach. *Ital J Gastroenterol* 1994;26:7–11.