Specific Serum Immunoglobulin G Response to Urease and CagA Antigens of Helicobacter pylori in Infected Children and Adults in a Country with High Prevalence of Infection

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Received 8 June 2001/Returned for modification 13 September 2001/Accepted 4 October 2001

Few studies have analyzed the immune response to Helicobacter pylori CagA and urease antigens across age groups in the same population. The aim of this study was to analyze the serologic immunoglobulin G (IgG) response to CagA and urease in children and adults with gastrointestinal symptoms and belonging to the same population and similar socioeconomic levels. The serologic response was studied in 352 children and 293 adults with gastrointestinal symptoms. IgG antibodies against CagA and urease were tested by enzyme-linked immunosorbent assay methods using highly purified recombinant antigens. H. pylori infection was defined as a positive result in a serologic assay using whole-cell H. pylori extracts as the antigen. We found, in H. pylori-positive children, a seroprevalence of 46.9% to CagA and 16.2% to urease, whereas in H. pylori-positive adults, a seroprevalence of 78.9% to CagA and 59% to urease was found. In children, the magnitude of the response to CagA was significantly higher and the response to urease was significantly lower than those in adults. The kinetics of the serologic response to CagA and to urease across age groups was contrastably different. Whereas CagA is a strong immunogen, urease is a poor immunogen during natural infection. These differences in the humoral response may be important for the short-term or long-term outcome of the infection. These results add to our knowledge of the epidemiology of H. pylori infection.

At present, it is well accepted that infection with Helicobacter pylori may lead to duodenal and gastric ulcers, mucosa-associated gastric lymphoma, and distal gastric cancer in humans (5). The infection is usually acquired during childhood, although expression of disease does not occur in most cases until adulthood. The presence of the organism causes gastric inflammation in all individuals; with time, this chronic inflammation may lead to injury of the gastric mucosa (23). Inflammatory mediators, such as interleukin-8 and interleukin-1, alter physiologic functions, such as gastric acid secretion. The infection during childhood is often transitory, and spontaneous eradication can be seen (27). In contrast, this phenomenon is rarely observed in adults. The reasons for this difference are unclear, but the nature of the inflammatory and immune responses may partially explain the phenomenon. In particular, the immune response to some antigens in H. pylori infection in children may differ from the response observed in adults. It has been reported that after infection, children mount an immune serologic response primarily to low-molecular-weight antigens (24); this contrasts with the serologic response observed in adults, in whom antibodies against both low- and high-molecular-weight proteins are observed. These results suggest that during the acute phase of the infection, the host responds mainly to low-molecular-weight antigens, and that it may take months or even years to mount a detectable serologic response to other antigens (19). These differences in the humoral response to H. pylori infection may be important for the short-term outcome of infection in children, such as spontaneous eradication, or the long-term outcome in adults, such as development of gastrointestinal diseases.

There are a rather large number of reports studying the humoral immune response to H. pylori whole-cell extract or to a pool of semipurified antigen preparations in individuals of different ages in the same populations (3, 12, 15, 20, 21, 24, 25, 26, 28, 29). These studies have been useful for learning the prevalence of the infection across age groups; however, few studies have analyzed the humoral response to purified antigens in individuals of all ages in the same population. In particular, there are few studies (2, 4, 6) that have determined the natural immune response to urease, a prominent vaccine candidate that has been studied in the animal model (7). The aim of this study was to analyze the serologic immunoglobulin G (IgG) response to two of the most important antigens of H. pylori, CagA and urease proteins, in symptomatic children and adults of the same population and similar socioeconomic levels. These antigens were chosen because CagA is a marker for the presence of the cag pathogenicity island and urease is an enzyme responsible for counteracting gastric acidity, allowing H. pylori colonization of the gastric mucosa.

MATERIALS AND METHODS

Patients. The pediatric population studied included 352 children who were seen at the Gastroenterology Unit of the Pediatric Hospital (Centro Médico Nacional SXXI-IMSS) because of gastrointestinal symptoms; of this group, 242 had recurrent abdominal pain (based on Appleby’s criteria), 89 presented with non-ulcer dyspepsia (NUD) (abdominal discomfort), and in 21 children the clinical diagnosis was unknown. The adult population consisted of 293 persons seen at

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TABLE 1. Serum IgG responses to whole-cell extract, CagA, and urease in children and adults according to clinical diagnosis

| Age group and diagnosis | No. of patients studied | No. (%) positive for IgG antibodies against: |
|-------------------------|-------------------------|--------------------------------------------|
|                         |                         | Whole cell | CagA | Urease |
| Children                |                         |            |      |       |
| RAP                     | 242                     | 86 (35.5)  | 40 (46.5)%* | 9 (10.5)%** |
| NUD                     | 89                      | 31 (34.8)  | 21 (67.7)  | 8 (25.8)   |
| Adults                  |                         |            |      |       |
| PUD                     | 143                     | 129 (89.6) | 108 (83.7)%*** | 72 (55.8) |
| NUD                     | 150                     | 133 (86.7) | 99 (74.4)  | 83 (62.4)  |

* P = 0.04, RAP versus NUD; ** P = 0.07, RAP versus NUD; *** P = 0.07, PUD versus NUD.

RESULTS

There were 645 subjects studied, including 352 children with a mean age of 9.5 ± 3.9 years and 293 adults with a mean age of 48.1 ± 15.9 years. *H. pylori* infection was detected by serology in 130 (36.9%) children, while among those infected, 61 (46.9%) had antibodies against CagA and 21 (16.2%) had antibodies against urease. In contrast, 261 (89.1%) of 293 adults were infected based on serology; among these, 206 (78.9%) had antibodies against CagA and 154 (59%) had antibodies against urease.

Clinical diagnoses were available for 331 children and for all 293 adults; response to whole-cell antigen, CagA, and urease in these patients is described in Table 1. In children, *H. pylori* infection was similar in both patients with recurrent abdominal pain (RAP) and patients with NUD; however, response to CagA was significantly higher in children with NUD. In adults, *H. pylori* infections determined by serology were similar in patients with PUD and with NUD; responses to CagA and urease were not significantly different in these two groups.

The magnitudes of the IgG responses to these antigens in infected children and adults are described in Table 2. IgG anti-CagA was significantly higher and IgG anti-urease was significantly lower in children than in adults. To further characterize this contrasting response, its magnitude was analyzed in different age groups (Fig. 1). It is important to mention that the highest response to CagA was observed in children (>7 ELISA units); response decreased steadily associated with age (to <5 ELISA units). In contrast, the magnitude of the response to urease was low in children (<3 ELISA units), subsequently increasing in young adults (>5 ELISA units) and decreasing in older individuals (<4 ELISA units).

Because of the difference in results observed between the

![FIG. 1. Magnitudes (in ELISA units) of the serologic IgG responses to CagA and urease in *H. pylori*-infected patients according to age.](http://cvi.asm.org/)

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TABLE 2. Magnitudes of IgG responses to CagA and urease in children and adults

| Age group | Magnitude of IgG response (ELISA units) |
|-----------|----------------------------------------|
| Children  |                                      |
| CagA      | 10.39 ± 6.94**                         |
| Urease    | 2.44 ± 1.42**                          |
| Adults    |                                      |
| CagA      | 6.94 ± 5.4                             |
| Urease    | 6.10 ± 3.28                            |

** All patients were seropositive for *H. pylori* infection by serology; ***, P < 0.001, children versus adults.
responses to CagA and urease, we compared the responses to urease in patients positive or negative for CagA antibodies (Table 3). Seropositivity to urease was higher in patients negative for CagA in both children and adults, but the difference was significant only in adult patients, although the magnitude of the anti-urease response in CagA-positive children and adults was not significantly different from that in individuals negative for anti-CagA antibodies.

**TABLE 3. Frequency and magnitude of IgG anti-urease response in patients infected with *H. pylori* with or without antibodies against CagA**

| Age group and anti-CagA status | $n$ | Response to urease | Magnitude (ELISA units) |
|-------------------------------|-----|--------------------|-------------------------|
|                               |     | No. (%) seropositive |                         |
| Children                      |     |                     |                         |
| Anti-CagA positive            | 62  | 8 (12.9)            | 3.3 ± 1.6               |
| Anti-CagA negative            | 68  | 13 (19.1)           | 2.3 ± 1.1               |
| Adults                        |     |                     |                         |
| Anti-CagA positive            | 208 | 114 (54.8)**        | 5.9 ± 3.3               |
| Anti-CagA negative            | 52  | 38 (73.1)           | 6.3 ± 3.3               |

$^a$ Infection was defined serologically, with a whole-cell antigen ELISA; **, $P < 0.01$, anti-CagA positive versus anti-CagA negative.

**DISCUSSION**

As expected, using the serologic IgG response to whole-cell antigens as the criterion to define infection, *H. pylori* infection was more frequent in adults than in children. Also, the frequency of the response to both CagA and urease antigens was significantly lower in children than in adults. When the clinical diagnosis was considered, children with NUD had a higher response to CagA and urease than children with RAP. It is probable that children with RAP represent patients with an acute infection, whereas children with NUD represent patients with a more prolonged infection, which explains the higher IgG response to *H. pylori* antigen preparations. In a previous report, we found similar results (2). An alternative hypothesis is that the poor immune response to *H. pylori* antigens in children with RAP may suggest that *H. pylori* has no association with RAP, as suggested by others (27).

In a study of schoolchildren in Estonia (29), a population with a high prevalence of *H. pylori* infection similar to that reported in our country (26), 46% of the infected children were seropositive to CagA. It should be noted that the criterion used for *H. pylori* infection in that study was exclusively serologic. When we used the same serologic criterion, a similar seropositivity to CagA (45%) was found. Interestingly, in a previous study of the same Estonian population (28), a higher seropositivity to CagA was found in adults (63%), also in accordance with our results, suggesting that *H. pylori* cagA-positive strains are more prevalent in adults than in children in both countries. In contrast, a study of symptomatic Japanese children (12) reported a seropositivity of >80% to CagA. However, >60% of these children had PUD, indicating that the criterion used in the selection of patients was different from that used in our study. It should also be noted that in Japan, the prevalence of cagA-positive *H. pylori* strains is very high, nearly 100%; in contrast to the results in our population, there was no difference between children and adults (12).

Other studies have also reported the seroprevalence to CagA in children when invasive tests are used to diagnose *H. pylori* infection. In France (24), in children positive by culture and histology, antibodies against CagA were detected in 43% of the infected children; in Israel (30), among children with *H. pylori*-positive culture and histology test results, 43% were seropositive for CagA. However, in Finnish children (14), seropositivity to CagA was 69% in patients positive by culture. In Italy (18), in a study using a rapid urease test and histology for *H. pylori* diagnosis, seropositivity to CagA in children was 43%. These values probably represent the actual prevalence of cagA-positive strains colonizing children in those populations, although the age of the patient may also influence the rate of seropositivity. In fact, in children *H. pylori*-positive by culture and histology in Brazil (25), response to CagA was 66% in those without ulcers; however, this seropositivity increased with age, from 12% in 2- to 6-year-old patients to 88% in those >12 years of age. These results agree with our findings and further suggest that the prevalence of cagA-positive strains has decreased in younger generations. In a recent report (9), we compared the presence of the cagA gene in strains isolated from children and adults and found that 47% of *H. pylori* isolates from children had the cagA gene, in contrast to 90% in *H. pylori* isolates from adults; thus, cagA genotyping agrees with serologic detection of CagA protein expression (46%).

There is, however, a proportion of children with serologic positivity for *H. pylori* as the only evidence for infection; these are probably children colonized with cagA-negative *H. pylori* strains, which further suggests the disappearance of cagA-positive strains in the younger generations.

An alternative explanation is that immune response to CagA requires more time than response to other antigens, such as in those studies that demonstrated that in children the initial response is mounted mainly against low-molecular-weight proteins and that it may take months to see a response to high-molecular-weight proteins, including CagA (24, 25). Another possibility is the spontaneous eradication of *H. pylori* in children (1, 13, 22); these children would be seropositive for *H. pylori* infection but negative for immune response to CagA.

An intriguing finding was that the magnitude of the IgG response to CagA was higher in children and that it decreased with age; this result contrasted with the magnitude of the response to urease, which increased steadily with age and reached a maximum in patients >60 years of age. These results suggest that the kinetics of the immune responses to CagA and urease are contrastably different. The contrasting responses to CagA and to urease are further documented with our finding that the serologic response to urease is significantly higher in both children and adults seronegative for CagA. These observations are difficult to interpret; one possibility would be that cagA-negative strains express more urease, offering a higher antigenic challenge to the gastric mucosa.

In the present study, we found that serologic IgG response to urease is low in children (16%) and that this response increases with age, although only 60% of adults developed IgG anti-urease antibodies. The magnitude of the serologic response also increased with age. These results suggest either that during a natural infection urease is a weak immunogen or that expression of this antigen is gradually increased during the infection and that years of colonization are required to induce an immune response. In previous studies, we have reported...
similar results in both symptomatic patients and a community-based population (2, 15). The results of the present study further document our previous findings. In a recent study of young adults (average age, 26 years) (8), no serologic IgG response to urease was detected in patients with gastritis. These results are in accordance with our previous findings of a low response to urease in young adults.

Several authors have suggested that urease is highly immunogenic and have even proposed its use for diagnosis of the infection (4, 11). However, the majority of these studies have used partially purified urease, which probably contains other more potent antigens, such as heat shock proteins (6). A possible explanation for these results may be related to the type of antigen used in the serologic assays. In all our studies (2), we have used a highly purified recombinant antigen that is devoid of other \emph{H. pylori} antigens. In addition, we have previously shown that there is a good correlation between the recognition of recombinant urease in ELISA and the recognition of native urease in Western blot analysis (2); this suggests that recombinant urease is a good antigen for the study of the IgG response to urease.

In conclusion, our results demonstrate that there are differences between the serologic responses to CagA and urease in children and adults. The low response observed in children may confirm the gradual decline in colonization with the most virulent \emph{H. pylori} strains in the community, particularly in younger generations. The observation that children with RAP had a lower IgG response to CagA and urease than children with NUD suggests that RAP may not be associated with \emph{H. pylori} infection. The kinetics of response to CagA and urease are contrastably different: in the natural infection, apparently CagA is a strong immunogen and urease is a poor immunogen.

ACKNOWLEDGMENTS

This work was supported by the Coordinación de Investigación Médica, IMSS, and by Conacyt (Mexico) grant 28040.

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