NOTE

Age-Specific Immune Response to HspA in Helicobacter pylori-Positive Persons in Mexico

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The immune response to heat shock protein A (HspA) in Helicobacter pylori-positive adults increases with age in developed countries. This response has not been studied with children or in developing countries (G. I. Pérez-Pérez, J. M. Thiberge, A. Labigne, and M. J. Blaser, J. Infect. Dis. 174:1046–1050, 1996). As determined by using a specific enzyme-linked immunosorbent assay, HspA seropositivity among 592 individuals in Mexico was <10% in children and increased to >40% in adults.

It is known that Helicobacter pylori is responsible for eliciting a remarkable array of immunologic responses in the gastric mucosa, involving adhesion molecules, chemokines, and cytokines, suggesting a predominant Th1 immune response (3), which eventually may lead to disease.

HspA and HspB are two heat shock proteins that have been described for H. pylori. While HspB is similar to other bacterial GroEL homologs, the HspA antigen possesses a unique histidine-rich C-terminal domain that other GroES homologs lack. This highly charged, nickel-binding domain of the HspA protein may play an essential role in the assembly of the urease-nickel complex (4, 5).

Preliminary evidence with the mouse model suggests that DNA vaccines encoding HspA or HspB decrease gastric mucosal inflammation (10), but the clinical significance is uncertain. One possibility is that the HspA immune response could be a marker for histological damage. HspA seropositivity has been associated with gastric cardia inflammation in H. pylori-positive patients but not with esophageal disease or adenocarcinoma of the cardia (7). However, the lack of correlation between a particular level of antibody to HspA and the histological findings has been reported (1).

Previous studies have shown that HspA seropositivity in H. pylori-positive subjects ranges from 39 to 60% (6, 7, 9, 12). Two of these studies (6, 9) demonstrated that HspA seropositivity increased gradually with age. All of these studies involved adult subjects and were performed in developed countries. Thus, we sought to determine HspA seroprevalence in a developing country, Mexico, and whether there was a relationship between HspA seropositivity, gastrointestinal disease, and/or patient age.

Presumably, H. pylori-negative sera cannot be HspA positive; therefore, we used them to determine the immune response background of HspA. To achieve this goal, we performed HspA enzyme-linked immunosorbent assays (ELISAs) with 31 children (mean age, 8.7 ± 3.9 years) who were all negative for H. pylori by serology, culture, and histology. Those children sought medical attention for recurrent abdominal pain (RAP). We are aware that H. pylori is not a relevant etiological cause for RAP, but we are including them because, based on their symptoms, they are eligible for upper endoscopy. The 31 H. pylori-negative children with RAP provided the opportunity to determine the immune response background of HspA immunoglobulin G (IgG). This would subsequently allow us to determine the threshold value for HspA.

TABLE 1. Association between age and HspA seropositivity among 592 H. pylori-positive persons, by clinical status

| Age group (yr) | Asymptomatic | Symptomatic | All |
|---------------|--------------|-------------|-----|
|               | HspA seropositive | HspA seropositive | HspA seropositive |
|               | No. of samples | % HspA seropositive | No. of samples | % HspA seropositive | No. of samples | % HspA seropositive |
| 0–19          | 172          | 9.3         | 23    | 17.4         | 195         | 10.3         |
| 20–39         | 162          | 22.8        | 15    | 13.3         | 177         | 22.0         |
| 40–59         | 101          | 46.5        | 29    | 24.1         | 130         | 41.5         |
| ≥60           | 67           | 43.0        | 23    | 39.1         | 90          | 42.2         |
| Total         | 502          | 25.7        | 90    | 24.4         | 592         | 25.5         |

a HspA seropositivity was defined as a net optical density value greater than the predetermined threshold value.

b H. pylori positive by serologic criteria.

c Symptomatic indicates duodenal ulcers in adults or RAP in children.

d Chi-square for trend, P < 0.001.

e Chi-square for trend, P = 0.23.

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seropositivity. Given the high prevalence of *H. pylori* in Mexico, it was logically easier to document *H. pylori*-negative status in this childhood population. The main study population included 502 asymptomatic persons and 90 dyspeptic *H. pylori*-positive persons. Of this population, there were 136 children and 366 adults, between the ages of 3 and 98 years (mean age, 32.4 ± 20.1). The asymptomatic population was part of the National Serologic Survey in Mexico, as previously reported (11). The symptomatic population included 68 consecutive *H. pylori*-positive adults with duodenal ulcer (mean age, 51.5 ± 17.0 years) and 22 consecutive *H. pylori*-positive children with RAP (mean age, 9.8 ± 3.6 years). All symptomatic patients sought medical attention at the Mexican Institute for Social Security in Mexico City. To further the analysis, each symptomatic patient was matched according to gender and age (±3 years) to an asymptomatic subject. In total, 89 of 90 symptomatic and asymptomatic patients were matched.

*H. pylori* status for both symptomatic adult and child patients was determined by serology, culture, and histologic biopsy, as previously reported (13). *H. pylori* status for asymptomatic persons was determined exclusively by serology. To assess IgG antibodies to *H. pylori* whole-cell antigens, an antigen-specific ELISA was performed, as previously reported (2, 8). The whole-cell antigens were derived from the sonicated pool of three *H. pylori* strains isolated from Mexican patients. To assess IgG antibodies to HspA, we used our previously described ELISA (9). The IgG immune response to HspA was determined in parallel ELISAs using maltose binding protein (MBP) alone and MBP-HspA as coated antigens, with patient sera diluted 1:100 in duplicated wells. The two optical density values for MBP were averaged and subtracted from the average of the two optical density values for MBP-HspA, yielding the net optical density value (NOD) that we report here. To determine the HspA threshold value, we added three standard deviations to the calculated mean of the 31 *H. pylori*-negative children. We defined HspA seropositivity as having an NOD value greater than or equal to the threshold value (0.069).

We determined the serological responses to HspA in 502 asymptomatic individuals and 90 symptomatic *H. pylori*-positive patients, stratifying the groups by age (Table 1). HspA seropositivity was 25.7 and 24.4% for the asymptomatic and symptomatic populations, respectively. Both groups showed increasing seropositivity with age. However, a significant correlation between HspA seropositivity and age was observed only among the asymptomatic population (P < 0.001; chi-square test for trend) or when both groups were analyzed together (P < 0.001; chi-square test for trend).

Another observation was that the mean NOD value for HspA-seropositive persons in the asymptomatic population was 0.33, whereas the mean NOD value for the HspA-seropositive persons in the symptomatic population was 0.48 (P = 0.007; Student’s t test). However, there was no significant trend in the intensity of the immune response to HspA with age (Table 2).

Among the 89 age- and gender-matched pairs of symptomatic and asymptomatic individuals (Table 3), HspA seropositivity correlated with age in asymptomatic individuals (P = 0.01; chi-square for trend), but in the symptomatic subjects age did not correlate with HspA seropositivity (P = 0.16; chi-square for trend). However, similar to the nonmatched population, the mean NOD of the symptomatic subjects was significantly higher than the mean NOD of the asymptomatic group (0.48 versus 0.25; P < 0.001; Student’s t test).

Our findings confirm that the immune response to HspA among symptomatic and asymptomatic *H. pylori*-positive indi-

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**TABLE 2. Correlation between age and intensity of the serological response to HspA among 151 HspA responders with or without symptoms**

| Age group (yr) | Asymptomatic | Symptomatic | All HspA Seropositives |
|---------------|--------------|-------------|-----------------------|
|               | No. of samples | Mean NOD ± SD | No. of samples | Mean NOD ± SD | No. of samples | Mean NOD ± SD |
| 0–19          | 16           | 0.26 ± 0.2   | 4           | 0.54 ± 0.4   | 20           | 0.32 ± 0.3   |
| 20–39         | 37           | 0.33 ± 0.2   | 2           | 0.66 ± 0.5   | 39           | 0.33 ± 0.2   |
| 40–59         | 47           | 0.38 ± 0.3   | 7           | 0.30 ± 0.2   | 54           | 0.27 ± 0.2   |
| ≥60           | 29           | 0.31 ± 0.2   | 9           | 0.56 ± 0.2   | 38           | 0.37 ± 0.2   |
| Total         | 129          | 0.33 ± 0.2ₕ  | 22          | 0.48 ± 0.3ₖ  | 151          | 0.35 ± 0.3   |

ₕ NOD, net optical density.  
ₖ Comparing asymptomatic versus symptomatic HspA-seropositive persons, P = 0.007 (Student’s t test).

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**TABLE 3. Matched⁶ comparison between 89 asymptomatic and 89 symptomatic *H. pylori*-positive persons in Mexico**

| Age group (yr) | Asymptomatic persons | Symptomatic persons |
|---------------|----------------------|---------------------|
|               | No. of samples | % HspA seropositive | Mean NOD* of HspA among seropositives, ± SD | No. of samples | % HspA seropositive | Mean NOD* of HspA among seropositives, ± SD |
| 0–19          | 23           | 8.7             | 0.08 ± 0.0           | 23           | 17.4            | 0.54 ± 0.4           |
| 20–39         | 15           | 20.0            | 0.21 ± 0.1           | 15           | 13.3            | 0.67 ± 0.5           |
| 40–59         | 29           | 41.4            | 0.24 ± 0.1           | 29           | 24.1            | 0.30 ± 0.2           |
| ≥60           | 22           | 50.0ₕ           | 0.28 ± 0.2           | 22           | 40.9ₕ           | 0.56 ± 0.2           |
| Total         | 89           | 31.5            | 0.247 ± 0.10ₖ         | 89           | 24.7            | 0.484 ± 0.30₇        |

ₕ Matched by age (±3 years) and by gender.  
ₖ P = 0.01 (chi-square for trend).  
₇ P = 0.16 (chi-square for trend).  
₇ Comparing asymptomatic and symptomatic HspA-seropositive persons, P < 0.001 (Student’s t test).  
₇ NOD, net optical density.
individuals in Mexico correlates significantly with age, as previously reported for developed countries (6, 7, 9, 12). This study supports the hypothesis that seroreponsiveness to HspA begins during early childhood. If *H. pylori* strains universally express HspA both in vivo and in vitro, the reasons why only a small proportion of colonized persons mount a detectable immune response to this antigen remain unclear.

The results of our study indicate that there is no correlation between patients with duodenal ulcer or RAP and the serological response to HspA. However, this may be the result of an insufficient number of participants in the gender- and age-matched comparison. Nevertheless, our findings were similar to those of previous studies that did not show a significant relationship between the presence of *H. pylori*, clinical outcomes, and HspA seropositivity (6, 9). Additional studies involving a larger population would further assess the relationship between different disease states and the serological response to HspA.

This study, in combination with other studies in other parts of the world, indicates that the increase in seropositivity to HspA with respect to age is not restricted to any particular ethnicity, region, or country development status. The exact role of HspA and the host response to HspA have yet to be elucidated. A further understanding of the immunological response to HspA will aid in our understanding of the pathogenesis of *H. pylori* as a chronic infectious agent.

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