B-Cell and T-Cell Immune Responses to Experimental *Helicobacter pylori* Infection in Humans

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The acute antibody and T-cell immune response to *Helicobacter pylori* infection in humans has not been studied systematically. Serum from *H. pylori*-naive volunteers challenged with *H. pylori* and cured after 4 or 12 weeks was tested by enzyme-linked immunosorbent assays for anti-*H. pylori*-specific immunoglobulin M (IgM) and IgA established using bacterial lysates from homologous (the infecting strain) and heterologous *H. pylori*. Proteins recognized by IgM antibody were identified by mass spectrometry of immunoreactive bands separated by two-dimensional gel electrophoresis. Mucosal T-cell subsets (CD4, CD8, CD3, and CD30 cells) were assessed by immunohistochemistry. All 18 infected volunteers developed *H. pylori*-specific IgM responses to both homologous or heterologous *H. pylori* antigens. *H. pylori* antigens reacted with IgM antibody at 4 weeks postinfection. IgM Western blotting showed immunoreactivity of postinfection serum samples to multiple *H. pylori* proteins with molecular weights ranging between 9,000 (9K) to 150K with homologous strains but only a 70K band using heterologous antigens. Two-dimensional electrophoresis demonstrated that production of *H. pylori*-specific IgM antibodies was elicited by *H. pylori* flagellins A and B, urease B, ABC transporter binding protein, heat shock protein 70 (DnaK), and alkyl hydroperoxide reductase. Mucosal CD3, CD4, and CD8 T-cell numbers increased following infection. IgM antibody responses were detected to a range of homologous *H. pylori* antigens 2 to 4 weeks postchallenge. The majority of *H. pylori* proteins were those involved in motility and colonization and may represent targets for vaccine development.

The important human pathogen *Helicobacter pylori* causes a persistent gastroduodenal infection that produces a brisk humoral and cellular immune response. The histological characteristics of the mucosal inflammation contain features of both acute and chronic inflammation. Although much is known about the clinical manifestations of chronic *H. pylori* infection, there is little information regarding the immune response in the early phases of infection (11, 39). One major obstacle to the study of the early events in *H. pylori* infection in humans has been the difficulty in determining when an individual actually becomes infected. As such, the majority of the literature related to the immune response to the early phases of *H. pylori* infections has been extrapolated from data acquired from the screening of populations for the presence of anti-*H. pylori* immunoglobulin M (IgM), IgA, and IgG antibodies and from a few cases in which the acquisition of the infection was known with reasonable certainty (1, 2, 9).

The initial humoral immune response to most bacterial infections involves a humoral IgM response. However, the available data regarding an IgM response among cases of acute *H. pylori* infection are both infrequent and inconsistent. For example, follow-up of two cases of acute *H. pylori* infection in adults reported no serologic IgM response at any time. However, one of the cases showed a local mucosal IgM response within the gastric mucosa at day 14 (18, 43). That patient developed a detectable serum *H. pylori*-specific IgG response by day 74 postinfection (43). A study of a family with intrafamilial transmission of *H. pylori* infection reported an IgM response in both the children and a parent (37). Their index case was an infant with a history of vomiting that settled spontaneously. The patient’s sibling was diagnosed with acute *H. pylori* infection 9 days later, based on histology. An IgM response was noted in both children that peaked at day 9 in the index case and rose over the first 63 days in the sibling. The infection was subsequently transmitted to their father, in whom a specific IgM was noted by day 63. Both children had a detectable serum anti-*H. pylori* IgG by day 30, whereas the father developed a serum IgG response between days 209 and 259. Finally, a serologic IgG response in one of the two reported cases of self-inoculation with *H. pylori* was noted between 22 and 33 days postingestion and was preceded by an IgM response (38).

Longitudinal studies of the humoral immune response in several groups of children have also been reported (8, 19, 45). Czinn et al. noted *H. pylori*-specific IgM in 19% of symptomatic children with active *H. pylori* infection (8). A follow-up study of 80 Taiwanese infants showed that three of six Taiwanese infants with naturally acquired *H. pylori* infection developed a short-lived IgM response preceding development of an IgG antibody response (19). Finally, Gambian children were tested at 3-month intervals, and a rise in anti-*H. pylori* IgM antibodies was noted around the time of the first positive urea breath test.
(UBT) (12). In those children, the IgG response was delayed until approximately 9 months after the first positive UBT (45).

In 1999, we initiated studies aimed at establishing an experimental H. pylori infection in humans that could be used for future vaccine studies. These studies were based on the long history of clinical trials in which subjects are vaccinated and subsequently challenged to determine the protective activity of vaccine. Such candidates have been used in the development of vaccines and drugs against enteric and respiratory infections such as malaria, Q fever, cholera, Norwalk virus, rhinoviruses, influenza virus, dengue viruses, sand-fly fever virus, and respiratory syncytial virus and infections with Salmonella enterica serovar Typhi, enterotoxigenic Escherichia coli, Shigella, and Campylobacter jejuni (4, 5, 23, 42, 44, 46). Details of the study design and results have been published elsewhere (20).

The current study examined the antibody and T-cell immune response to acute H. pylori infection including data regarding kinetics, type, and duration of the humoral and cellular immune responses and the specific H. pylori proteins eliciting the response after an infection of known onset and duration. We also report the effects of using both homologous versus heterologous H. pylori strains as the antigen sources for antibody testing.

MATERIALS AND METHODS

A cag pathogenicity island-negative, OipA-positive, multiple-antibiotic-susceptible strain of H. pylori obtained from an individual with mild gastritis (Baylor strain 100 or ATCC BAA-945) was used for the oral challenge of 20 volunteers (9 women and 11 men, ages 23 to 33 years). Volunteers received 40 mg of famotidine at bedtime and 104 to 105 CFU of H. pylori in beef broth the next morning. Infection was confirmed by 13C-UBT, culture, and histology. Antibiotic eradication therapy was given 4 (n = 12) or 12 (n = 4) weeks postchallenge, and eradication was confirmed by at least two separate UBTs, as well as culture and histology. Eighteen volunteers (90%) became infected. Mild to moderate dyspeptic symptoms occurred, peaked between days 9 and 12, and resolved. Gastric histology obtained 2 weeks postchallenge showed typical chronic H. pylori gastritis with intense acute and chronic inflammation. The density of H. pylori (as assessed by CFI/biopsy) was similar independent of the challenge dose (20).

Following challenged developed a humoral immune response.

Antigen source. H. pylori strains ATCC 43504 (heterologous) and BCS-100 (homologous) were used in enzyme-linked immunosorbent assays (ELISAs) and Western blots. The BCS-100 challenge strain was genotype cagA negative and positive for vacA s1c-m1, iceA2, bbalA2. It contained a functional OipA protein, as there was no frameshift in the 5′ region of the oipA gene. The organism was susceptible to amoxicillin, tetracycline, metronidazole, and clarithromycin. The susceptibility of the specific antibody response was identified based on the OD value of the baseline control serum with each case serving as their own control. A positive result was an OD value above the baseline OD value.

Serum samples from volunteers or from known H. pylori-negative and H. pylori-positive subjects were serially diluted twofold starting at 1:50 and were added to both antigen-positive and antigen-negative wells. The negative and positive control sera used in all subsequent assays were selected from serum samples tested for the presence of anti-H. pylori IgM. Serum samples were incubated for 2 h at 37°C following overnight incubation at 4°C. Mouse anti-human IgM labeled with peroxidase (Southern Biotechnology Associates, Birmingham, AL) (100 μl) diluted 1:2,500 in blocking buffer was added to each well and incubated for 1 h at 37°C. The specificity and sensitivity of the secondary antibody was confirmed against human IgG (Chemicon, Temecula, CA), human IgA (Cappel, Livermore, CA), human secretory IgA (sIgA) (Chemicon, Temecula, CA), and IgM (Cappel, Livermore, CA) by coating microtiter plates with the human antibodies that were serially diluted twofold starting at 10 μg/ml with 0.078 μg/ml being the last dilution on the plate. No reactivity was detected when the conjugate used in this ELISA was tested against human IgG. When the secondary antibodies were tested against human IgA and human sIgA, the detection limits were 0.15 μg/ml and 1.25 μg/ml, respectively. Finally, when the secondary antibodies used in this ELISA were tested against human IgM, the detection limits were 5 μg/ml.

Serum IgA ELISA. Serum samples were collected from H. pylori-negative subjects at baseline, allowing each volunteer to serve as his/her own control. The ELISA optical density (OD) value at baseline was subtracted from ELISA OD values of all subsequent postchallenge serum samples. An IgM H. pylori-specific antibody response was identified based on the OD value of the baseline control serum sample with each case serving as their own control. A positive result was an OD value above the baseline OD value.

Preparation of H. pylori antigen-coated microtiter plates. The optimal concentration of each reagent used in the ELISA was determined by checkerboard titration. Alternating wells of flexible polyvinyl chloride microtiter plates (Dynex Technologies, Inc., Chantilly, VA) were coated with 100-μl well H. pylori antigen (7.5 μg/ml) diluted in 0.01 M PBS (antigen-positive well) or 0.01 M PBS (antigen-negative well). The plates were incubated at 37°C for 2 h. The plates were washed between every step with 200 μl of 0.05% Tween 20 in 0.01 M PBS using an Ultrawash Plus plate washer (Dynatech Laboratories, Inc., Chantilly, VA). Following washing, all wells were blocked by the addition of 200 μl of blocking buffer (10% powdered skim milk in 0.01 M PBS) at room temperature for 2 h and used for one of the following ELISAs.

Serum IgM ELISA. Serum samples were collected from H. pylori-negative and positive subjects at baseline, allowing each volunteer to serve as his/her own control. The ELISA optical density (OD) value at baseline was subtracted from ELISA OD values of all subsequent postchallenge serum samples. An IgM H. pylori-specific antibody response was identified based on the OD value of the baseline control serum sample with each case serving as their own control. A positive result was an OD value above the baseline OD value.

Western blot analyses. Volunteer serum samples were tested for reactivity to H. pylori proteins by Western blotting as described previously, with slight modification (40). Briefly, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli with a 0.75-mm-thick 10% separating gel and a 4% running gel using a Mini Protein II Cell apparatus (Bio-Rad Laboratories, Hercules, CA). Solution A consisted of 5.98 g of Tris and 0.46 ml of TEMED (tetramethylethylenediamine) in 100 ml of distilled water adjusted to a pH of 6.6 with hydrochloric acid. To prepare the loading buffer, 5 ml of 0.5 M sodium dodecyl sulfate, 1 ml of 3 M glycerol, 1 ml of diethylpyrocarbonate, 100 μl of 0.5% phenol red, 2.5 mM 2-mercaptoethanol, and 0.6 g of sodium dodecyl sulfate were mixed and heated at 95°C for 10 min. One microgram of either homologous or heterologous H. pylori (freeze-thawed) antigen or 10 μl of protein ladder (Bench Mark prestained protein ladder; Gibco BRL) were loaded per lane, and the electrophoresis was...
The gel pieces were then rehydrated in 0.1 M NH₄HCO₃ containing 0.5 to 1
NH₄HCO₃/50% acetonitrile for
analysis in the reflector mode on an Applied Biosystems Voyager DE-STR MALDI-
(alpha-cyano-4-hydroxycinnamic acid) spotted and dried again, followed by analy-
sis in the reflector mode on an Applied Biosystems Voyager DE-STR MALDI-
TOF mass spectrometer. Monoisotopic peptide masses detected were sent to
ProFound (PROWL, Rockefeller University) or MS-FIT (Protein Prospector, University of California, San Francisco) for protein database searches and pro-
tein identification by peptide mass fingerprinting.

Immunohistochemistry. Formalin-fixed sections of biopsy specimens of the
gastric antrum were processed by the immunohistochemistry service in the De-
partment of Pathology at the University of Texas Medical Branch, Galveston,
as previously described (3). Briefly, the biopsy specimens were obtained from study
participants undergoing gastroesophageal endoscopy during the current study, as
approved by the institutional review boards at Baylor College of Medicine and
the University of Texas Medical Branch. The T-cell distributions were measured
prior to the challenge and at 4 weeks postchallenge. T cells in three zones (neck,
pit, and gland) were counted in seven (7/18) infected volunteers. Three of these
volunteers were infected for 4 weeks and four were infected for 12 weeks.
Adjacent sections were stained using an automated staining process in which they
were labeled with antibodies recognizing human CD3, CD4, CD8 (Ventana,
Tucson, AZ), CD50 (Dako Corp., Carpinteria, CA), or an appropriate immu-
noglobulin isotype control (Ventana). Slides were counterstained with hematox-
ylin and eosin. Tissue sections were examined by two readers who did not have
prior knowledge of the stain, although the state of infection was self-evident. Cell
counts are expressed as the number of positive cells in five high-powered fields.

Statistical analyses. For the ELISAs, the mean OD values and standard
deviations were determined and compared using the Student t test, which was
used to calculate a P value using the STATA statistical software package (Col-
lege Station, Texas). T-cell counts were compared using the Wilcoxon rank sum
test.

RESULTS

Anti-H. pylori IgM response. Because the ability to detect serum IgM responses can be compromised if other Ig isotype
antibodies are also present in a sample, we tested whether the
presence of IgG to H. pylori interfered with the detection of anti-
H. pylori IgM. Anti-H. pylori IgG-positive and anti-H.
pylori IgM-negative or anti-H. pylori Ig total-negative and anti-
H. pylori IgM-positive serum samples were mixed in equal
volumes to a final dilution of 1:50, and IgM and total antibody
titers were compared with the results of the unmixed sample.
The H. pylori-specific IgM titers were identical for the unmixed
and mixed IgM-positive samples, indicating that anti-H. pylori
Ig total antibodies did not interfere with anti-H. pylori-specific
IgM detection (data not shown).

The volunteers from both groups, infected for 4 weeks and
infected for 12 weeks, developed systemic anti-H. pylori
IgM antibodies (Fig. 1). All infected volunteers had detectable an-
ti-H. pylori antibodies by 4 weeks postchallenge. In general,
the ELISA OD values of serum samples collected from volunteers
infected for 12 weeks were higher compared to the OD values of
serum samples collected from volunteers infected for 4
weeks, but the difference was not statistically significant. H.
pylori-specific IgM antibodies were detectable as early as 2
weeks in 22% of volunteers.

Comparison of results between heterologous and homolo-
gous H. pylori antigen by ELISA. Anti-H. pylori IgM responses
were identified by ELISA in all 18 infected volunteers, irre-
spective of the antigen used. While the OD values of H. pylori-
specific IgM antibodies were higher with homologous than
with heterologous antigen, the difference was not statistically
significant. In addition, the kinetics of the cumulative serocon-
version rates was similar irrespective of the antigen used (Fig.
2) and the duration of the IgM response after H. pylori erad-
ication was also similar (data not shown). The H. pylori-specific
IgM antibodies persisted longer (17.7 weeks) after the curing

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of volunteers infected for 12 weeks compared to those infected for only 4 weeks (9.7 weeks).

H. pylori-specific IgA and IgG responses. To examine whether IgA antibodies developed during acute H. pylori infection, we tested serum samples from the three infected volunteers with the highest IgM antibody OD values at all time points (pre- and postchallenge) for H. pylori-specific IgA antibodies. H. pylori-specific IgA antibodies were not detected in any of the three volunteers tested (data not shown). We found that a specific anti-H. pylori IgG response began to appear about 4 weeks postchallenge and peaked 12 to 19 weeks postchallenge. Of note, 33% (n = 12) of the volunteers infected for only 4 weeks failed to develop IgG antibodies against H. pylori infection (data not shown).

Western blotting. We used Western blotting to examine which H. pylori proteins were responsible for eliciting H. pylori-specific IgM antibody. Sera from volunteers who developed H. pylori-specific serum IgM as detected by ELISA were tested against both the homologous and the heterologous H. pylori antigens by Western blotting (Fig. 3). Antibodies present in all sera collected before challenge recognized proteins with molecular weights (MW) of 50 and 55, and these proteins were considered to be cross-reactive antigens (32). In addition to these cross-reactive antigens, IgM antibodies recognized a single heterologous H. pylori protein with a MW of 70,000 (70K). Sera tested with the homologous strain of H. pylori recognized multiple proteins with MW of 9, 19 to 40, 60, 70, and 150K. While the frequency of reactivity to the higher-molecular-weight proteins was variable, all of the infected volunteer sera recognized a wide range of lower-molecular-weight H. pylori proteins with MW of 19 to 40K (Table 1). Reactivity to low-molecular-weight proteins (e.g., MW 19 to 40K) was detected by 2 weeks postchallenge in 11 of the 18 volunteers. By 3 to 4 weeks postchallenge, the immune response broadened to include recognition of additional proteins with MW of 9, 19 to 40, 60, 70, and 150K. IgM immunoreactivity of volunteer sera to heterologous and homologous H. pylori proteins peaked at approximately 4 weeks postchallenge. Generally, the intensity of bands increased from 2 through 4 weeks postchallenge and then remained approximately the same through 10 weeks postchallenge. Infection for 12 weeks was associated with a higher intensity and longer duration of immunoreactivity than those infected for 4 weeks. In volunteers infected for 12 weeks, the bands were faint but detectable at 26 weeks postchallenge (14 weeks after eradication therapy). In volunteers infected for 4 weeks, loss of immunoreactivity was observed approximately 16 weeks after eradication therapy.
Identification of immunogenic proteins of *H. pylori*. We used two-dimensional gel electrophoresis and mass spectrometry to identify the most-prominent and well-separated bands detected by IgM Western blotting. Five proteins of higher molecular weight were identified as urease B, flagellin A and B, elongation factor TU (EF-TU), and the DNA K *H. pylori* protein (homolog of the heat shock protein). Four lower-molecular-weight *H. pylori* proteins were identified (Fig. 4 and Table 2), including alkyl hydroperoxide reductase (TsaA), hydrogenase expression protein (HypB), iron(III) ABC transporter periplasmic iron-binding protein, and superoxide dismutase (SodB).

**Cellular immune response.** We also evaluated the cell-mediated response to acute *H. pylori* infection. Immunohistochemistry to detect CD3, CD4, CD8, and CD30 surface antigens was performed on sections of biopsy specimens obtained prior to and following *H. pylori* infections. CD30 cells were not detected among the T cells in the gastric mucosa (Table 3). Staining with the isotype control antibody did not identify any immunoreactive cells. Specific antibodies for individual T-cell subsets confirmed previous reports that showed that infection with *H. pylori* was associated with a marked T-cell infiltrate in the epithelium as well as in the lamina propria (3). Cells that were positively stained for CD3, CD4, CD8, and CD30 were counted, and there was a statistically significant increase in all T-cell subsets examined, including the CD4 and CD8 subsets. These changes were most dramatic in the neck but were generally found throughout the gastric glandular structure (Table 3).

**DISCUSSION**

*H. pylori* challenge studies provided a unique opportunity to study some aspects of the *H. pylori*-host interactions from the time of colonization and establishment of the infection through the early phases of the infection, including the B- and T-cell immune response. We examined the kinetics and duration of the *H. pylori*-specific IgM response following infections lasting...
TABLE 2. Identity of proteins that elicited production of IgM antibodies during the early phase of H. pylori infection

<table>
<thead>
<tr>
<th>MW</th>
<th>Protein identity</th>
<th>No. of peptides matched</th>
<th>% Sequence coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Alkyl hydroperoxide reductase (TsaA)</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>26</td>
<td>Superoxide dismutase (SodB)</td>
<td>13</td>
<td>75</td>
</tr>
<tr>
<td>32</td>
<td>Hydrogenase expression/formation protein (HypB)</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>34</td>
<td>Iron(III) ABC transporter, periplasmic iron-binding protein (CeuE)</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>47</td>
<td>EF-TU</td>
<td>20</td>
<td>62</td>
</tr>
<tr>
<td>56</td>
<td>Flagellin A</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>58</td>
<td>Flagellin B</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>65</td>
<td>Urease beta subunit (urea amidohydrolase)</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>67</td>
<td>DnaK protein (heat shock protein 70)</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

more than several individuals with known initiation of infection. Based on previous reports, IgM responses in infected adults and children were inconsistent; few IgM seroconversions were observed in children (19%), and onset of appearance after presumed exposure differed greatly (from few weeks to 3 months) (8, 37, 38, 45). Our study, in which the onset of the infection was known with certainty, showed that up to 80% of adults infected with H. pylori developed an acute IgM antibody response within 2 to 4 weeks of infection and that the response could persist for more than 4 months after eradication of the infection. In children, IgM seroconversion to H. pylori has been reported for 19 to 50% of patients. However, the numbers of children tested were small and the onset of disease was rarely known. Although this alone may account for differences with our results, alternatively, the IgM responses might differ between children and adults. We also had the advantage of being able to use homologous antigens which resulted in an increase in reactivity (i.e., optical density). We collected serum samples regularly, which allowed for more accurate monitoring of IgM antibody development, and we found that the H. pylori-specific IgM antibodies peaked at 4 weeks postinfection and fell within 2 to 4 months after beginning of eradication therapy. In contrast, the anti-H. pylori IgG response began to appear about 4 weeks postchallenge and peaked 12 to 19 weeks postchallenge. These data are similar to the experimental inoculation of H. pylori in chimpanzees, where IgG seroconversion occurred at 3 weeks with a further rise of H. pylori-specific IgG antibodies by week 11 after the challenge (21). Rhesus monkeys have also been reported to develop seroconversion 2.5 to 3.5 months postchallenge (10).

H. pylori proteins from the homologous H. pylori strain that elicited anti-H. pylori IgM antibodies in the volunteers included proteins with molecular weights of 19 to 40, 60, 70, and 150K. Previous reports of proteins recognized by H. pylori-specific IgM antibodies include proteins with MW of approximately 31K, 60K, 67K, and 90K (7, 16, 48). However, as the identity of the proteins was not determined, a direct comparison between studies of differences in proteins recognized by IgM is not possible. The majority of volunteers showed a response to the lower-molecular-weight proteins, which is similar to the pattern described following H. pylori infection of Mongolian gerbils (30). In contrast to the response with homologous antigens, only one H. pylori protein band with a MW of 70K reacted with volunteers’ sera when heterologous antigen

TABLE 3. Anti-H. pylori T-cell response in seven infected volunteers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Median T-cell numbers (range)*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
</tr>
<tr>
<td>CD3</td>
<td>9.2 (4.8–18.2)</td>
</tr>
<tr>
<td>CD4</td>
<td>0.6 (0.2–1.6)</td>
</tr>
<tr>
<td>CD8</td>
<td>4.6 (2.6–14.4)</td>
</tr>
<tr>
<td>CD30</td>
<td>0.2 (0–2)</td>
</tr>
</tbody>
</table>

* n = 7; * P value < 0.05. Data are divided by the indicated sites and by relation to infection (pre- or postinfection). The T-cell distributions were measured prior to the challenge and at 4 weeks postchallenge. T cells in the three zones (neck, pit, and gland) were counted (n = 7) as described in Materials and Methods. The increase of CD3, CD4, and CD8 cells is statistically significant as assessed by the Wilcoxon rank sum test. CD3 marker reacts with T cells associated with the T-cell antigen receptor; CD4 marker reacts with a subset of T cells that carry the coreceptor protein CD4 that activates macrophages and B-cell responses to antigen; CD8 marker reacts with cytotoxic CD8 cells that recognize antigens; CD30 marker reacts with a tumor necrosis factor receptor family member that enhances B-cell proliferation.
was used for Western blotting. The current study does not allow us to distinguish whether the 70K band represents one or several proteins of similar molecular weight. However, overall the immunoreactivity obtained with heterologous antigen was clearly restricted compared to that obtained using the homologous antigen. This difference is consistent with the organisms marked ability to undergo genetic rearrangements which may reduce the cross-reactivity between immunogenic epitopes on the homologous and heterologous *H. pylori* proteins (24, 25).

Because Western blotting allows only presumptive identification of *H. pylori* proteins, we used two-dimensional gel electrophoresis and mass spectrometry to identify proteins that were prominent and well separated. Because of these limitations, we did not attempt to identify all of the protein bands identified by Western blot analysis using sera obtained 4 weeks after inoculation. Nine *H. pylori* proteins that elicited production of IgM antibodies were identified (Table 2). These proteins can be characterized as being generally involved in colonization and establishment of *H. pylori* infection. For example, flagella are thought to be essential for the ability of *H. pylori* to colonize the gastric mucosa (26). FlaA, elongation factor EF-TU, and urease B have all been reported to be highly immunoreactive proteins (34). In addition, sera from volunteers also recognized heat shock protein 70 (35). We identified four lower-molecular-weight proteins, including hydrogenase expression/formation protein, known to be a housekeeping enzyme involved in energy metabolism; alkyl hydroperoxide reductase, involved in general cellular processes; superoxide dismutase, involved in combating host defenses; and iron(III) ABC transporter periplasmic iron-binding protein, which is likely important in iron homeostasis in what is generally an iron-poor environment. The hydrogenase expression protein and alkyl peroxidase reductase are among the immunodominant antigens of *H. pylori* based on reactivity to serum collected from patients infected with *H. pylori* infection (28). IgM antibody recognized additional *H. pylori* proteins that were not separated well enough to be clearly identified. Additionally, our use of antigens prepared in vitro may have limited the expression of important proteins and/or virulence factors expressed in vivo (e.g., host-bacteria cell contact is absent) and may have lead to an underestimation of the range of proteins eliciting the IgM immune response (28). Nonetheless, we were able to identify and characterize a number of antigens recognized in early infection.

The pattern of T-cell subsets infiltrating the gastric mucosa is essentially identical to those observed in natural infection of unknown duration (3, 15). For example, cells expressing CD30 are rarely found in the gastric mucosa after natural infection, and they were also essentially absent from the mucosa of the volunteers after *H. pylori* challenge. Moreover, most of the T cells accumulated in the neck of the gastric gland. In previous reports, CD4-positive cells increased much more than CD8 cells (3). In this acute phase of the infection, this difference was less remarkable, and this may reflect the differences in a chronic versus a subacute infection as well as variation in patient populations, techniques, or the infecting strain. It is also possible that the relative contribution of intraepithelial lymphocytes is increased and, if so, this could inflate the percentage of CD8+ T cells. The current study did not separate the component of intraepithelial lymphocytes that would contribute to the CD8 counts, as only mucosal T-cell populations were recorded. We chose to assess total mucosal T-cell counts in an effort to avoid selective sampling and because of the difficulty in discerning the relationship of the T cells to the tortuous shape of the epithelial glandular unit. Nonetheless, the T-cell responses in these subjects as defined by their surface antigen expression are an excellent model of the changes observed in response to natural infection.

The ontogeny of the cellular immune response to infection is often extrapolated from the observed response to immunization. CD4-positive helper T cells would be expected to expand in the first 42 to 72 h. Thereafter, the expression of various chemokines would recruit CD4- and CD8-bearing T cells. Our results suggest that the major changes in T-cell subset recruitment and expansion have occurred by 4 weeks postinfection.

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