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Serum Antibody Response to the Superficial and Released Components of *Helicobacter pylori*

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Superficial and released components were extracted from six selected *Helicobacter pylori* strains. The protein and antigenic profiles of these extracts were representative of the profiles found most frequently among the clinical strains and included major peptidic fractions at 19, 23.5, 57, 68, 76, 118, and 132 kDa and major antigens at 68, 57, and 23.5 kDa. Immuno-cross-reactions were seen with a hyperimmune rabbit serum to *Campylobacter fetus* but not with sera to *Campylobacter jejuni* or *Salmonella* spp. An antigenic preparation was obtained by pooling equivalent quantities of each extract, and the antigenic preparation was used to study the antibody responses of sera from 65 French patients and 127 Tunisian patients. By enzyme-linked immunosorbent assay, we observed that the sera from French and Tunisian patients clustered into two populations, defined as antibody positive (72 patients) and antibody negative (120 patients). The antibody-positive patients were more frequently infected with *H. pylori* ($P < 0.01$) and were more frequently affected with gastritis ($P = 0.05$). However, no correlation between antibody levels and clinical signs of dyspepsia was noticed. The proportions of antibody-positive patients were similar in France and Tunisia. Antibody-positive and antibody-negative sera were studied by Western blot (immunoblot) analysis. The antibody-positive sera revealed an average of 7.7 antigenic bands, whereas the antibody-negative sera revealed an average of 2.4 antigenic bands ($P < 0.01$). The antigens between 15 and 40 kDa and greater than 66 kDa were specifically recognized by the antibody-positive sera, although in this molecular size range the antibody profiles of these sera exhibited a fairly high degree of diversity. We conclude that the superficial and released components from *H. pylori* contain a variety of bacterial immunogens and may be useful in antigenic preparations for the serodiagnosis of *H. pylori* infections. Moreover, a group of antigens in combination appears to be useful for discriminating antibody-positive and antibody-negative patients.

Helicobacter pylori is a newly recognized organism associated with gastroduodenal pathology (3, 4, 12). This organism specifically colonizes the gastric mucosa, mainly because of its particular adaptation to the gastric environment. This adaptation is due in particular to the release of high amounts of urease (8) and also to the adhesion factors that allow bacterial attachment to the gastric mucosal cells (6, 9, 11, 31). Despite a strong local and systemic immune response, *H. pylori* infection is persistent, and up to now, the reason for this persistence was unknown (23); therefore, it may be of interest to improve our knowledge of the human immune response to this organism.

The serum antibody response to *H. pylori* has been studied in particular in order to develop serologic methods of diagnosis. Those studies have been conducted by using either purified bacterial components or crude bacterial extracts as antigens (2, 10, 13, 17, 20, 21, 26–28, 30). Although the crude antigenic preparations cross-react with antigens from closely related bacteria, they are among the preparations more frequently used to achieve these serologic reactions (15, 29). These antigenic preparations include certain superficial components and most of the parietal components. The performances of the serologic assays are usually evaluated on the basis of their sensitivities, specificities, and predictive values. To calculate these parameters, two sets of clearly defined antibody-positive and antibody-negative sera are needed. Such defined sera should ideally be obtained from two distinct groups of subjects: (i) subjects known to have been infected with *H. pylori* and (ii) subjects known to be naive regarding *H. pylori* infection. Unfortunately, such characteristics are difficult to assert on a practical basis, and usually, *H. pylori* carriage is determined on the basis of various reference methods of diagnosis considered to be "gold standards." This raises the issue of the choice of a gold standard which would enable us to discriminate *H. pylori*-positive and -negative subjects. Culture of *H. pylori* from biopsy specimens or the [^{13}C]urea breath test (19) are the most often used reference methods of diagnosis; however, both tests may give false-negative results, and for now, there is no real consensus for such a gold standard. Consequently, it is possible to arrive at quite different performance values for a given serologic method, depending on the reference method used to define the immune status of the subjects (29).

Characterization of the most immunogenic *H. pylori* components is also interesting. One would think that the major immunogens are exposed on the surface of the bacterial cell. Actually, several investigators have highlighted an abundant loose material surrounding *H. pylori* cells (11, 14). This external material is easily extracted and contains a variety of antigenic bacterial components, including the urease subunits (7), most of the bacterial hemagglutinins, and most of the bacterial adhesins (6, 11, 14). During natural infection, this superficial material containing components which play major roles in the pathogenesis of *H. pylori* infection may be located at the interface between the mucosa-adhering bacteria and the host cells (14). This material may consequently act as a major immunogen.

The aim of the work described here was to study the proteins and the antigenic components of the superficial material and

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the material released from *H. pylori* and to use them as antigens in serological assays to assess the immunologic status of patients.

MATERIALS AND METHODS

Strains and extraction of the superficial material of *H. pylori*. The six bacterial strains used in the present work (H92-1, US-456, H88-M, TX30-A, H92-2, H92-4) were isolated in France, Tunisia, or the United States from gastric biopsy specimens from patients suffering from gastritis. They were chosen because their protein profiles were representative of the most frequently encountered protein profiles of the clinical strains examined. Their protein profiles were stable from one culture to another. These strains were kept in nutrient broth no. 2 (Oxoid) with 10% glycerol and were frozen at −80°C until use.

Superficial bacterial material was extracted from cultivated *H. pylori* cells as described previously (11). Briefly, the bacteria were thawed out and cultured on Columbia blood agar (Bio-Mérieux, Marcy l'Etoile, France) for 4 days at 37°C under microaerophilic conditions. Bacterial cells were harvested and suspended in phosphate-buffered saline (PBS; pH 7.4). The original suspension was vigorously vortexed for 1 min, 100 μl was saved for further determination of viability, and the suspension was centrifuged (5,000 × *g*) for 10 min. The supernatant (superficial material) was saved, filtered on a 0.22-μm-pore-size microfilter, adjusted to 1 mg of protein per ml, and frozen at −20°C until use. The pellet was resuspended (final suspension) in 2 ml of PBS, and the mixture was gently vortexed. Smears from both the original and the final suspensions were prepared and Gram stained to check that the bacterial cells retained their morphologic and staining characteristics after extraction. Furthermore, viable counts of the original and the final suspensions were performed to check that the extraction did not lead to a loss of viability. Each new preparation was checked to determine that it had protein and antigenic profiles identical to those of the original preparation.

Patients and human sera. One hundred ninety-two patients (age range, 1 to 72 years; median, 21 years) were included in the study, which was conducted between January 1989 and January 1993. Sixty-five patients were from France (Boucicaut Hospital, Paris, or University Hospital, Poitiers) and 127 patients were from Hôpital Charles Nicolle (Tunis). These patients were outpatients of internal medicine departments; 144 of them were clinically documented for gastric pathology and were distributed into the following four clinical categories: (i) nondyspeptic children (15 years old and younger), (ii) dyspeptic children, (iii) nondyspeptic adults, and (iv) dyspeptic adults. Of these 144 patients, 107 underwent upper gastroduodenal endoscopy and biopsy for histologic and bacteriologic examinations. Blood samples were obtained from the patients on the day on which they were examined, and the sera were rapidly frozen at −20°C. Subjects who had been treated over the 4 previous weeks with antiacid or antibiotic medications were excluded from the study.

Laboratory methods. Histologic sections of biopsy specimens were stained with hematoxylin-eosin and Giemsa. They were then observed for histologic signs of gastritis (18) and the presence of spiral-shaped organisms. Each biopsy specimen was also used to prepare Gram-stained smears to look for curved gram-negative bacilli and was also cultured under microaerophilic conditions on Columbia blood agar (Bio-Mérieux) at 37°C for 5 days. *H. pylori* was identified by conventional laboratory methods (14).

Rabbit sera. For preparation of anti-*H. pylori*, anti-*Campylobacter fetus*, anti-*Campylobacter jejuni*, and anti-*Salmonella* spp. sera, 500 μg of protein from sonicated specified bacteria or superficial material from the bacteria was given subcutaneously to rabbits with the same volume of complete Freund adjuvant. After the administration of two booster shots, the rabbits were bled (6 weeks after the original injection) and the sera were kept at −20°C until use.

Immunochemical analysis of superficial material from *H. pylori*. Protein determinations were performed by the bicinchoninic acid method (25). The protein profiles were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) by the method of Laemmli (17a) with 12% acrylamide running gels and 4% acrylamide stacking gels. Samples were denatured for 5 min at 96°C in 1% SDS, and the gels were loaded with 1 to 5 μg of protein. Electrophoretic migrations were carried out for 2 h at 25 V/m. For determinations of protein profiles, the gels were silver stained and submitted to analysis by using a picture analyzer (Bio-Profil, Vilber-Lourmat, France). For Western blotting (immunoblotting), the gels were electrotransferred onto nitrocellulose and were revealed by using various appropriately diluted serum specimens. The blots were revealed with an appropriate alkaline phosphatase-conjugated serum specimen. The percent homologies between the protein or antigenic profiles were determined by the method of Dice (5).

Titration of antibodies by ELISA. A mixture of the superficial material from the six selected *H. pylori* strains was prepared and used as antigen (Hp-Ag). Microtiter plates (Immulon II; Dynatech) were coated with the Hp-Ag (1 μg per well) for 18 h at 4°C in 0.05 M carbonate buffer (pH 9.6). Excess binding sites of the plates were blocked for 6 h at 4°C with 1% bovine serum albumin (BSA) in 0.15 M PBS (pH 7.4) containing 0.05% (wt/vol) Tween 20 (Sigma) and 0.01% (wt/vol) thimerosal (Sigma) (PBS-TT), and the plates were washed three times with PBS-TT. The sera were diluted at 1/500 in PBS-TT containing 0.5% (wt/vol) rabbit gamma globulin (Sigma) and 0.1% (wt/vol) gelatin (Sigma) and were added to the plates (100 μl per well). The plate was then incubated for 1 h at 37°C and washed in PBS-TT, and 100 μl of a peroxidase-conjugated goat anti-human immunoglobulin G (IgG) preparation appropriately diluted in PBS-TT containing 0.1% (wt/vol) rabbit gamma globulin and 1% (wt/vol) BSA was added to each well. After 1 h of incubation at 37°C, the plate was again washed in PBS-TT and was developed for 30 min as described previously (11). The optical density (OD) obtained by the enzyme-linked immunosorbent assay (ELISA) was read on a microplate reader (Labtek Instruments, Salzburg, Austria) at 410 nm. The assay was calibrated by measuring, under the same conditions, the OD obtained by ELISA of a reference human serum specimen included on each plate. The ELISA index was the ratio of the OD of the studied serum specimen to the OD of the reference serum specimen. Each serum specimen and the reference serum specimen were assayed in triplicate.

Immunogold labelling. Bacteria (strain H92-1) were routinely cultured on blood agar for 4 days. Bacterial cells were harvested and suspended in 0.15 M NaCl with 4% formaldehyde. After 15 min at room temperature, they were washed three times in PBS (pH 7.4)–1% BSA–0.5% gelatin (PBS-BSA-gel). A grid covered with Formvar and carbon-coated film was immersed for 30 min in the bacterial cells that were suspended in PBS-BSA-gel. The grid was then immersed for 2 h in the hyperimmune rabbit serum raised to superficial material of *H. pylori* that was diluted to 1/10 in PBS-BSA-gel, and the grid was then rinsed three times in PBS-BSA-gel. The grid was then immersed in PBS-BSA-gel containing 10% goat

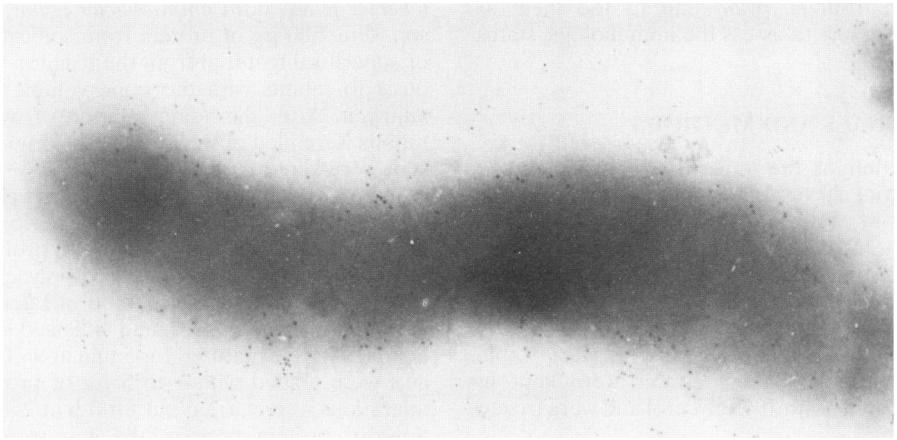

FIG. 1. Immunogold labelling of *H. pylori* H92-1 with hyperimmune rabbit serum to superficial material extracted from *H. pylori* strains. Magnification, ×33,000.

anti-rabbit IgG conjugated to 10-nm colloidal gold particles (Amersham, Les Ulis Cédex, France). After 1 h of incubation, the grid was rinsed in PBS-BSA-gel, rinsed again in distilled water, and examined on an electron microscope.

RESULTS

Evidence that the extracted material is surface exposed. Extraction of the superficial material did not lead to significant modifications of the shape of the bacterial cells as checked on Gram-stained smears. The extraction led to a decrease of less than 5% in the number of viable bacteria.

The rabbit hyperimmune serum raised to *H. pylori* material extracted from the six selected *H. pylori* strains was used to localize this material on the bacterial cell by immunogold labelling. The results are shown in Fig. 1. The strong labelling of the cell surface suggests that the majority of the antigens of the bacterial extract are exposed on the surface of the bacterial cell and that they may also be released out of the cell.

Immunochemical analysis of the superficial material from *H. pylori*. Superficial bacterial components were extracted from the six selected *H. pylori* strains. Under the experimental conditions that we used, most of the superficial components of *H. pylori* were extracted and the extracts contained the major part of both the urease activity and the superficial proteins that adhere to cultivated epithelial cells (7, 11). The protein profiles of the six corresponding extracts analyzed by SDS-PAGE are shown in Fig. 2. The homologies between these profiles ranged between 71 and 93% (mean, 80%; standard deviation [SD], 7%). The Hp-Ag resulting from the pooling of equivalent quantities of each individual extract exhibited all of the major fractions of the six extracts. Major bands were seen at 132, 118, 76, 68, 57, 23.5, and 19 kDa. The Hp-Ag was analyzed for the proportion of the different fractions. The resulting profile and the proportions of the different fractions are shown in Fig. 3. The largest amounts of proteins were located between 16 and 27 kDa (21.33%), 52 and 115 kDa (46.95%), and 127 and 137 kDa (5.43%).

The Hp-Ag was separated by SDS-PAGE and then immunoblotted to various hyperimmune rabbit sera. The results are shown in Fig. 4. By using the rabbit serum to Hp-Ag, the six strains (Fig. 4, lanes 1 to 6, respectively) exhibited similar antigenic profiles, with major antigens at 68, 57, and 23.5 kDa. The homologies of the six profiles ranged between 80 and 100% (mean, 88%; SD, 8%). The Hp-Ag was also blotted to sera raised to Hp-Ag (Fig. 4, lane 7), *C. fetus* 84-104 (Fig. 4, lane 8), *C. jejuni* 85H (Fig. 4, lane 9), and *Salmonella* spp. (Fig. 4, lane 10). The anti-*C. fetus* immunoreacted with Hp-Ag. Under the experimental conditions of the study, the other heterologous sera did not react with any fraction of Hp-Ag.

Serum antibody responses of 192 patients to the superficial antigens of *H. pylori*. The 192 human serum specimens were assayed by the ELISA for IgG antibodies to Hp-Ag. The distribution of the ELISA index was markedly skewed. Log transformation of the data corrected the skewness and revealed a bimodal distribution. The ELISA index for sera from both French and Tunisian patients showed a similar log-normal distribution (Table 1); this allowed us to consider both French and Tunisian sera as unique serum populations regarding antibodies to *H. pylori*. The distribution of the log ELISA index of all the 192 serum specimens is shown in Fig. 5. By using a one-dimensional cluster analysis based on euclidian distances between observations (Ward's minimum variance cluster analysis) (24), two subpopulations (median, −0.56; SD, 0.27; median, 0.17; SD, 0.19) clearly stand out from this distribution (Fig. 5). Although, the two subpopulations partially overlapped, the cutoff value appeared to be −0.12

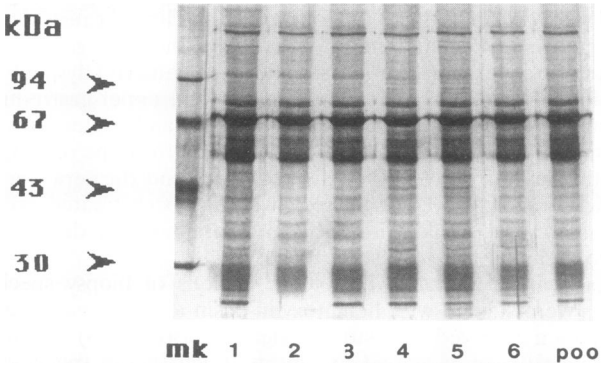

FIG. 2. Protein profiles of the superficial material extracted from six *H. pylori* strains and the mixture of the six extracts. Lane 1, strain H92-1; lane 2, strain US-456; lane 3, strain H88-M; lane 4, strain TX30 A; lane 5, strain H92-2; lane 6, strain H92-4; lane mk, molecular size markers; lane pool, profile of the pooled extracts (Hp-Ag). SDS-PAGE was performed on 12% acrylamide running gels and 4% stacking gels. The gels were silver stained.

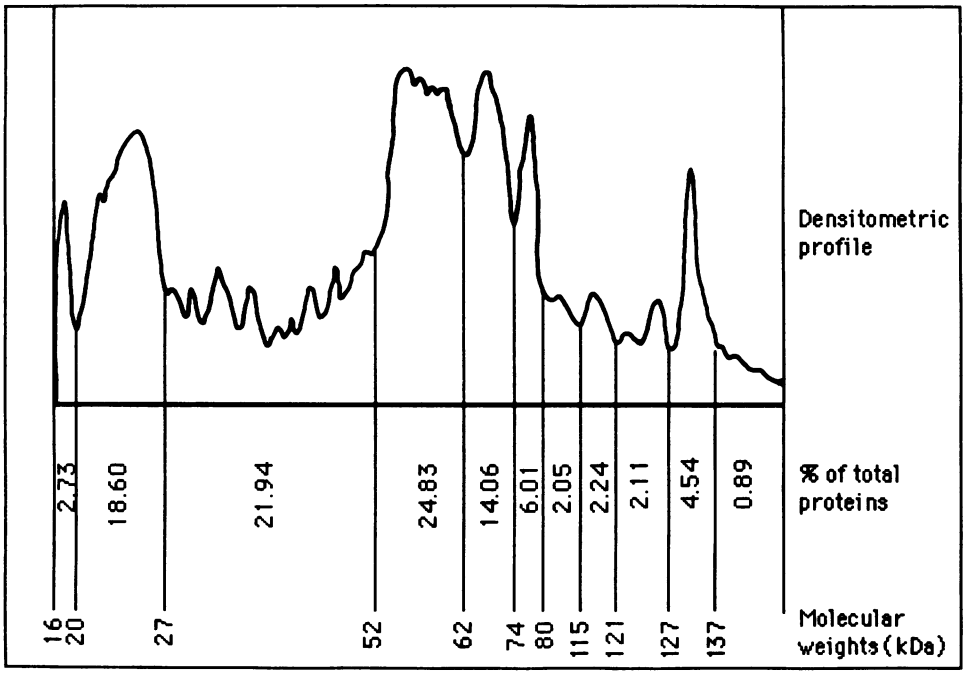

FIG. 3. Analysis of the protein profiles of the Hp-Ag resulting from the pooling of the superficial material extracted from six *H. pylori* strains. After SDS-PAGE (12% acrylamide) and silver staining, the gels were analyzed with a picture analyzer and the data were computed with the Bioprofil System (Vilmer-Lourmat, France) for the proportions of the different peptidic fractions.

(ELISA index, 0.75). Of the 192 patients, 120 had an index of less than 0.75 (antibody-negative patients) and 72 had an ELISA index of greater than 0.75 (antibody-positive patients). Of the 127 Tunisian patients, 46 (36.2%) were seropositive, and of the 65 French patients, 26 (40.0%) were seropositive. These proportions were not significantly different. Eight of the 59 children (13.5%) and 64 of the 133 adults (48.1%) were seropositive. These proportions were statistically different ($\chi^2 = 20.8$; $P < 0.01$).

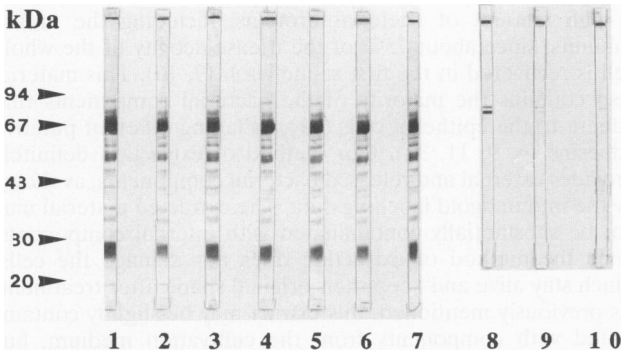

FIG. 4. Immunoblots of superficial material from six *H. pylori* strains with various hyperimmune rabbit sera. Bacterial extracts were separated by SDS-PAGE (12% acrylamide). Lanes 1 to 7, bacterial extracts from six *H. pylori* strains blotted to anti-*H. pylori* hyperimmune rabbit serum. Lane 1, strain H92-1; lane 2, strain US-456; lane 3, strain H88-M; lane 4, strain TX30 A; lane 5, strain H92-2; lane 6, strain H92-4; lane 7, mixture of the extracts from the six strains in lanes 1 to 6; lanes 8 to 10, mixture of the six extracts blotted onto hyperimmune rabbit sera raised to *C. fetus* (lane 8), *C. jejuni* (lane 9), and *Salmonella* spp. (lane 10).

In order to know the specificities of the *H. pylori* antibodies, antibody-positive and antibody-negative sera were diluted to 1/500 and blotted to the Hp-Ag already used for the ELISA. Three sets of blots are shown in Fig. 6: (i) 25 antibody-positive serum specimens from *H. pylori*-positive patients, (ii) 21 antibody-positive serum specimens from *H. pylori*-negative patients, and (iii) 26 antibody-negative serum specimens from *H. pylori*-negative patients. No antibody-negative sera were obtained from *H. pylori*-positive patients. The homologies of the antibody profiles of the antibody-positive sera ranged between 53 and 80% (mean, 72%; SD, 7%). These antibody-positive sera revealed an average of 7.7 bands (SD, 3.3 bands), whereas the antibody-negative sera revealed an average of 2.4 bands (SD, 1.3 bands). These two values were statistically different ($P = 0.0001$). Two groups of bands could be clearly distinguished. The bands of the first group (between 43 and 67 kDa) were revealed by both types of sera (although more intensively by the antibody-positive sera), whereas the other bands were especially revealed by the antibody-positive sera. Nevertheless,

TABLE 1. Characteristics of the ELISA index distributions of sera from Tunisian and French patients examined for antibodies against *H. pylori*

Cluster no.	Tunisian patients			French patients		
	No. of patients	Log ELISA index		No. of patients	Log ELISA index	
		Mean	SD		Mean	SD
1	81	−0.56	0.29	39	−0.59	0.21
2	46	0.17	0.19	26	0.17	0.22
Total	127			65		

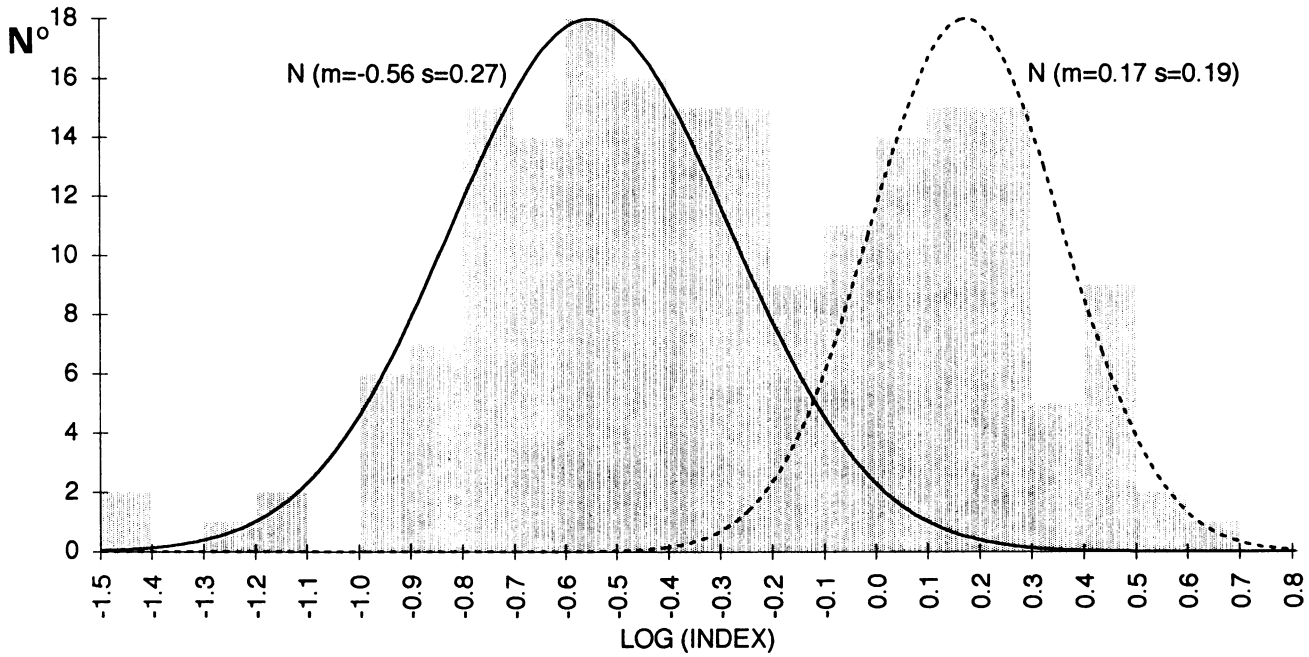

FIG. 5. Distribution of the log ELISA index of 192 serum specimens tested by ELISA for anti-*H. pylori* superficial antigens (bars). Microtiter plates were coated with 1 μg of Hp-Ag per well, and the sera were diluted to 1/500. The second antibody was goat anti-human IgG. Results of Ward's minimum variance cluster analysis of the log ELISA index distribution are also shown (lines). N, characteristics of the two clusters (m, median log ELISA index; s, standard deviation of the log ELISA index).

in this second group, the homology between the antibody profiles was low.

We established a correlation between the presence of *H. pylori* in the stomach (bacteriologically detected) and the presence of immunoreactive bands of less than 43 kDa on the blot. Of the 25 *H. pylori*-positive patients, 17 (68%) had antibodies to these antigenic fractions, whereas 8 of 47 (16%) of the *H. pylori*-negative patients had antibodies to these antigenic fractions. These proportions were significantly different ($\chi^2 = 19.2$; $P < 0.001$). On the other hand, 24 of 25 (96%) *H. pylori*-positive serum specimens exhibited immunoreactive bands of greater than 43 kDa, whereas 25 of 47 (52%) *H. pylori*-negative serum specimens exhibited such bands. These proportions were also significantly different, even though 52% of the *H. pylori*-negative sera immunoreacted in this range.

Correlations between clinical, histologic, or bacteriologic features and immunologic status. Of the 59 children (age 15 years and younger), 52 were clinically evaluated for dyspeptic syndrome. The same was true for 92 of the 133 adults (Table 2). Of the 52 children, 24 were dyspeptic. Six (25%) of these 24 dyspeptic children were seropositive for Hp-Ag, whereas 2 (7.1%) of the 28 nondyspeptic children were seropositive. This difference was not statistically significant. On the other hand, 9 (42.9%) of the 21 nondyspeptic adults were seropositive, whereas 34 (47.9%) of the 71 dyspeptic adults were seropositive. These two proportions were also not statistically significantly different. These results demonstrate that there is no correlation between the clinical and the immune status of the patients.

One hundred seven patients underwent endoscopy, and biopsy specimens were taken. These biopsy specimens were examined for *H. pylori* and documented for the presence of gastritis on the basis of endoscopic and histologic examinations. Table 3 shows the correlations between the pathologic and immunologic status of the patients. *H. pylori*-positive patients and patients with gastritis were statistically more abundant among the antibody-positive patients than the antibody-negative patients.

DISCUSSION

H. pylori cells have a loosely superficial material which surrounds the bacteria. This material is especially abundant on the bacteria observed in vivo on the gastric mucosa (14). This superficial material is easily extracted from the cells by different methods, including washing of cells with water, saline, or isotonic buffers (11). The *H. pylori* superficial material contains a high amount of bacterial proteins, including the urease subunits, since about 75% of the urease activity of the whole cell is recovered in the first saline wash (7, 16). This material also contains the majority of the bacterial components that adhere to the epithelial cells (11), including different putative adhesins (6, 9, 11, 31). Our method of extraction definitely provides external and released bacterial components, as shown by the immunogold labelling data. The extracted material may not be substantially contaminated with internal components, since the method of extraction does not damage the cells, which stay alive and keep their original shape after treatment. As previously mentioned, this extract may be slightly contaminated with components from the cultivation medium, but these components are found in small amounts and they do not immunoreact with antibodies to *H. pylori* (11). The composition of the extracted material is close to that of a glycine extract but is more easily obtained and is richer in *H. pylori* components, particularly in the components released from the cells, which are absent from the other antigenic preparations already used for the detection of anti-*H. pylori* antibodies. Although the protein profiles of the extracts were very close from strain to strain, some strains lack certain protein fractions, as has been found with other types of extracts (20, 29). However, the

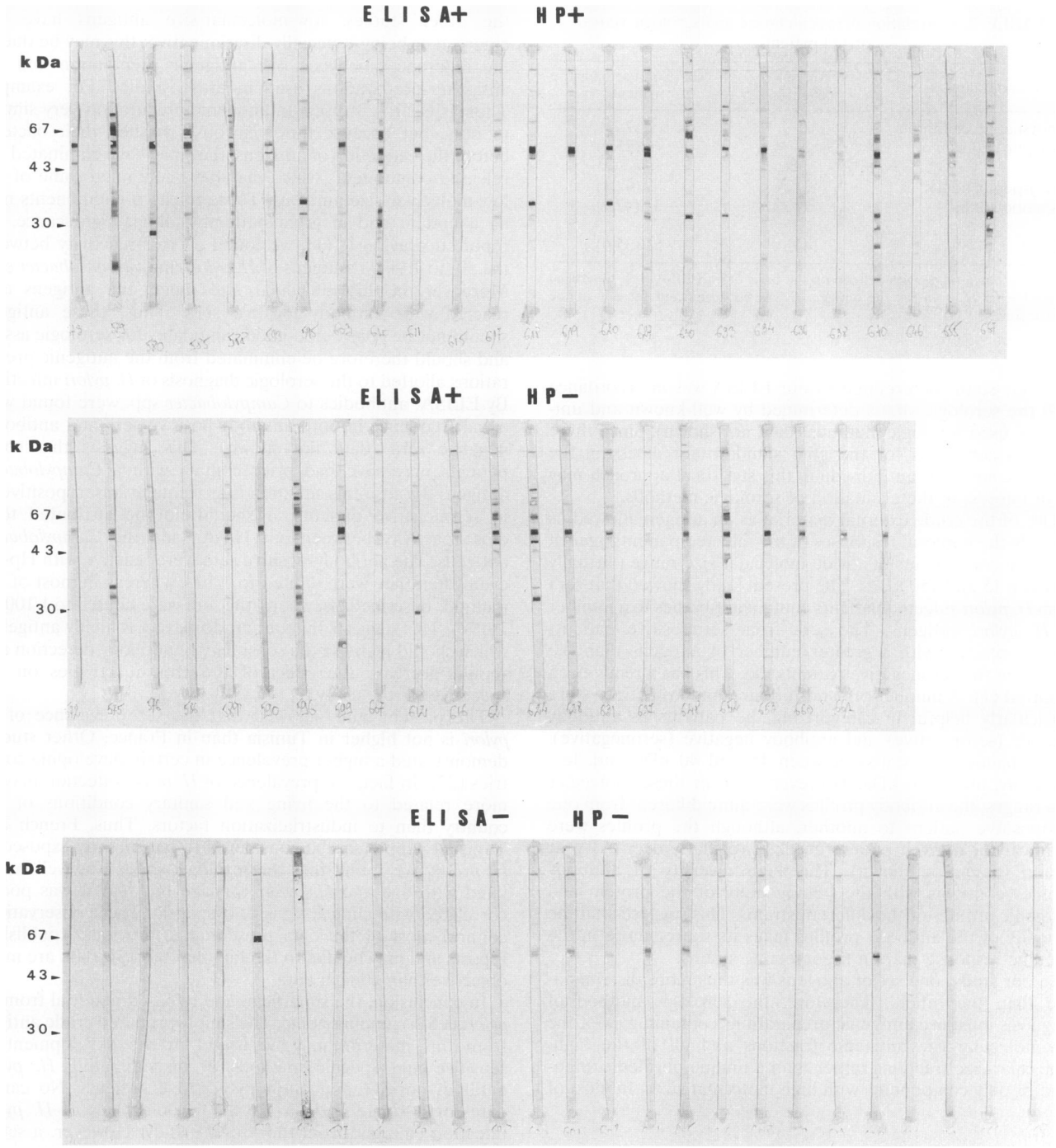

FIG. 6. Immunoblot of Hp-Ag to 25 antibody-positive serum specimens from *H. pylori*-positive patients (ELISA+, Hp+), 21 antibody-positive serum specimens from *H. pylori*-negative patients (ELISA+, Hp−) (last blot on the right is a duplicate of the previous one), and 26 antibody-negative serum specimens from *H. pylori*-negative patients (ELISA−, Hp−). Hp-Ag was separated by SDS-PAGE (12% acrylamide). Sera were diluted to 1/500, and the blots were developed with phosphatase-conjugated goat anti-human IgG serum.

major peptidic fractions present in our extracts have been also found in other types of extracts (1, 20, 29). These fractions include the flagellin, high-molecular-weight peptides, hemagglutinins, adhesins, and several unidentified outer membrane or released proteins. Thus, we assumed that the *H. pylori* antigenic preparation that we used in the present work is representative of the different superficial and released components found in different strains of *H. pylori*.

As expected, the *H. pylori* superficial material is highly antigenic and immunogenic. Using a statistical approach, we were able to cluster a priori two populations of patients (seropositive and seronegative) without any reference to clinical, bacteriologic, or histologic data. Nevertheless, it was possible a posteriori to show a statistical correlation between the patient's serologic status determined statistically and the carriage of *H. pylori* or the presence of gastritis. Moreover, the

TABLE 2. Correlation between clinical and serologic status of 144 patients

Clinical status	No. of patients	No. (%) of seropositive patients[a]
Nondyspeptic children	28	2 (7.1)
Dyspeptic children	24	6 (25.0)
Nondyspeptic adults	21	9 (42.9)
Dyspeptic adults	71	34 (47.9)
Total	144	51 (35.4)

[a] The serologic status was defined on the basis of an ELISA with superficial material from *H. pylori* as the antigen. *P* was not significant for any of the comparisons.

serologic status determined by our ELISA was in accordance with the serologic status determined by well-known and universally used serologic methods (data not shown). Since there is not a consensus for the gold standard for assessing the serologic status of an individual, this statistical approach may be of interest in the evaluation of serologic methods.

Use of the crude external material as an antigen allowed us to study the humoral responses of the infected patients against bacterial components in a wide molecular size range (virtually between 15 and 150 kDa). The present study showed that sera from *H. pylori*-infected patients contain antibodies to a number of *H. pylori* antigens. The sera from seropositive patients immunoreacted with a greater number of antigens than the sera from the seronegative patients did. This has already been reported (1). A number of bands of our standard antigen were particularly helpful in categorizing the patients as antibody positive (seropositive) and antibody negative (seronegative). These bands are located between 15 and 40 kDa and, less markedly, above 66 kDa. However, even in these molecular size ranges, the antibody profiles were quite different from one seropositive patient to another, although the profiles were identical for a given patient checked regularly over a 1-year period (data not shown). This high diversity of antibody profiles contrasts with the homogeneity of the protein and antigenic profiles of the different strains. This suggests that the diversity of the antibody profiles takes its source more in the immune response than in the infecting strains.

In our study, one set of antigens was thus more discriminative than the others. Therefore, the serologic method of diagnosis must use antigenic preparations containing all of the low-molecular-size antigenic fractions and all of the high-molecular-size fractions rather than a unique purified component or only components with high molecular sizes. In most of the other studies, low-molecular-size antigens have not emerged as being especially discriminative; this may be due to the differences between our antigenic preparation and the antigenic preparations used in those studies. For example, Thomas et al. (28) used an antigenic preparation very similar to ours, but because they vigorously washed their bacteria before the extraction of antigens, they may have eliminated the released components which may have contained some of the low-molecular-size antigens. These released components may be abundant and of great pathophysiologic significance. As reported previously (1), we found a cross-reactivity between the 50- to 70-kDa antigens of *H. pylori* and *Campylobacter* spp. Moreover, in this molecular size range, the antigens also reacted with antibody-negative sera. Thus, these antigens should not be considered good candidates for serologic assays and should therefore be eliminated from the antigenic preparations allotted to the serologic diagnosis of *H. pylori* infection. By ELISA, antibodies to *Campylobacter* spp. were found with equal frequency in both antibody-positive sera and antibody-negative sera (data not shown). This suggests that some patients may have had prior contact with a *Campylobacter* antigen. Because this situation is as frequent in seropositive as in seronegative patients, it should not be attributed to a cross-reactivity between our Hp-Ag and the *Campylobacter* antigens. The antibody-positive sera were reactive with Hp-Ag even when they were diluted to 1/500, whereas in most of the methods described elsewhere, the sera were diluted to 1/100 (1, 26, 28). This suggests that our crude extract is highly antigenic, which should increase the sensitivity of antibody detection and should decrease the effect of the cross-reactivities on the specificity of the assay.

TABLE 3. Correlation between pathologic and serologic status of 107 biopsied patients

Pathologic status	No. of patients	No. (%) of seropositive patients[a]	P (χ^2)
Presence of *H. pylori*[b]	38	25 (65.8)	0.001
Absence of *H. pylori*	69	21 (30.4)	
Gastritis[c]	89	42 (47.2)	0.05
No gastritis	18	4 (22.2)	

[a] Serologic status was defined on the basis of an ELISA with superficial material from *H. pylori* used as the antigen.
[b] Direct examination and culture of antral biopsy specimens.
[c] Endoscopic and histologic examination of the gastric mucosa.

The present study showed that the seroprevalence of *H. pylori* is not higher in Tunisia than in France. Other studies demonstrated a higher prevalence in certain developing countries (22). In fact, the prevalence of *H. pylori* infection may be more related to the living and sanitary conditions of the country than to industrialization factors. Thus, French and Tunisian people can be considered to be similarly exposed to *H. pylori*. We found that the serologic status was well correlated with age and *H. pylori* carriage but that it was poorly correlated with clinical signs of dyspepsia. These observations confirm most of the data presented in previously published reports and may be due to the fact that biologic data are more objective than clinical data.

In conclusion, the superficial and released material from *H. pylori* cells is immunogenic. The simply prepared crude antigen from this material may be useful in the development of sensitive and specific methods of discriminating *H. pylori* antibody-positive and antibody-negative patients. No candidate for a single antigen for the serodiagnosis of *H. pylori* infection emerged from the present study. However, a set of low-molecular-size antigens seems to be a good discriminative antigenic combination. Furthermore, a group of antigens of 43 to 66 kDa must be eliminated from the antigenic preparation in order to improve the specificity of serodiagnosis. Finally, the antibody response to *H. pylori* is very heterogeneous.

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