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Use of Serum-Specific Immunoglobulins A and G for Detection of *Helicobacter pylori* Infection in Patients with Chronic Gastritis by Immunoblot Analysis

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Multiple invasive and noninvasive tests for detecting *Helicobacter pylori* infection are available. The current "gold standard" for the diagnosis of *H. pylori* infection requires histology and the rapid urease test. Our aim was to test the performance of immunoglobulin A (IgA) and IgG immunoblot assays in comparison with that of the gold standard tests for the diagnosis of *H. pylori* infection. Ninety patients who underwent gastroscopy were analyzed in a prospective study. Fifty-nine of them were defined to be *H. pylori* positive by the gold standard tests. The IgA and IgG immunoblot assays correctly identified *H. pylori* infection in 17 and 58 of these patients, respectively, indicating that determination of IgA antibodies seems to be of low diagnostic value for *H. pylori* infection. In contrast, the sensitivity and specificity of the IgG immunoblot assay were 98 and 71%, respectively, with positive and negative predictive values of 87 and 96%, respectively. Therefore, the IgG immunoblot assay proved to be a sensitive and useful, noninvasive test for the diagnosis of *H. pylori* infection.

Helicobacter pylori plays an etiological role in the pathophysiology of various gastroduodenal diseases, in particular, type B chronic gastritis (16, 25, 28), duodenal and gastric ulcer (12), mucosa-associated lymphoid tissue lymphoma (23), and gastric adenocarcinoma (9, 22).

During the past few years, several methods for the simple and reliable diagnosis of *H. pylori* infection have been proposed. In principle, these methods are based on either invasive techniques which require endoscopy (e.g., biopsy, rapid urease test, culture, histology, and PCR) or noninvasive tests, such as the detection of antibodies to *H. pylori* or the exhalation of labelled CO_2 after ingestion of ^{13}C- or ^{14}C-urea (5, 18, 20, 24, 26).

Depending on the test that has been used, the detection of serum-specific antibodies of the immunoglobulin G (IgG) class has been shown to be sensitive (85 to 95%) and specific for determining *H. pylori* carrier status. Andersen (2) reviewed the results obtained with commercially available kits and reported that the positive predictive values were between 68 and 100% and the negative predictive values were between 53 and 99%. The detection of *H. pylori*-specific antibodies is consistent with a prolonged chronic mucosal infection, with IgA and IgG predominating (5). Since *H. pylori*-specific IgG antibody titers decline after bacterial eradication, serological tests can be useful for monitoring the success of therapy and as a screening test for epidemiological studies (20, 27).

The aim of our study was to evaluate the value of the immunoblot assay for determining *H. pylori*-specific IgA and IgG antibodies in serum samples from patients with chronic gastritis in comparison with the value of gastric histology and the rapid urease test.

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MATERIALS AND METHODS

Patients and specimens. Specimens were obtained from 90 dyspeptic patients; the patients included 51 males and 39 females, with an age range of 22 to 84 years. Patients were excluded if they had received antibiotics, nonsteroidal anti-inflammatory agents that are known to be risk factors for the development of peptic ulcer (17), or proton pump inhibitors within the previous 2 months or if they had experienced partial or complete gastrectomy. Six biopsy specimens were obtained from each patient during upper gastrointestinal endoscopy by using an Olympus GIF-100 HL videoendoscope (Olympus Optical, Hamburg, Germany). One antrum biopsy specimen and one corpus biopsy specimen were separately analyzed by a novel rapid urease test (HUT test; Astra Chemicals, Wedel, Germany). The biopsy specimens were evaluated for urease test positivity, as indicated by a color change from yellow to red, within 24 h. Two antral and two corpus biopsy specimens were fixed in 10% formalin and were embedded in paraffin. Sections (thickness, 5-μm) were stained with hematoxylin and eosin. The histological slides were reviewed by an experienced gastrointestinal pathologist blinded to the result of the rapid urease test and serology. *H. pylori* carrier status was defined when both histology and rapid urease test results were positive. Two milliliters of serum was obtained just before endoscopy and was stored at −20°C until further use for the immunoblot assay.

Immunoblot assay. The diversity of *H. pylori* strains has especially been described in relation to the expression of the vacuolating cytotoxin protein (VacA) and the cytotoxin-associated protein (CagA) (13, 30). In order to detect antibodies of diagnostic significance in the immunoblot assay, we used as a source of antigen *H. pylori* 151, which is known to express several antigens that are important for diagnosis, including the urease antigen (UreaA), VacA, and CagA (4, 14a, 15). Total bacterial cell lysate was obtained from a culture of this strain by centrifugation, washing in phosphate-buffered saline (PBS; 150 mM NaCl, 20 mM sodium phosphate [pH 7.2]), and subsequent resuspension in a buffer consisting of equal volumes of (i) PBS and (ii) 120 mM Tris (pH 6.8), 4% sodium dodecyl sulfate (SDS), 20% glycerol, 10% mercaptoethanol, and 0.002% bromphenol blue. After boiling for 5 min, the cell lysate was applied at a concentration of 20 μg per gel to SDS–11% polyacrylamide gels with subsequent blotting onto 0.45-μm-pore-size nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) as described previously (10). The nitrocellulose membrane was cut into strips, and each strip was incubated for 16 h with human serum diluted 1:100 in PBS, followed by 90 min of incubation with alkaline phosphatase-conjugated goat anti-human IgG diluted 1:8,000 or anti-human IgA diluted 1:4,000 (Dianova, Hamburg, Germany). Finally, reactive bands were visualized by using 5-bromo-4-chloro-3-indolylphosphate and incorporating nitroblue tetrazolium as the substrate. The positive and negative controls consisted of serum samples from either a known *H. pylori*-infected patient or a noninfected patient, respectively.

Statistical analyses. The chi-square test was used to evaluate the results.

RESULTS

By using SDS-polyacrylamide gel electrophoresis, the antigens of *H. pylori* were divided into three major groups: (i) low-molecular-mass proteins (LMMPs) ranging in mass from 22 to 33 kDa and including the 30-kDa urease antigen (UreaA), (ii) medium-molecular-mass proteins (MMMPs) ranging in mass from 43 to 66 kDa, and (iii) high-molecular-mass proteins (HMMPs) ranging in mass from 87 to 128 kDa and including the 87-kDa vacuolating cytotoxin protein (VacA) and the 128-kDa cytotoxin-associated protein (CagA). A serum sample was considered positive by the immunoblot analysis if antibodies against at least one HMMP or against at least two LMMPs could be detected (21).

Both histological examination of and the rapid urease test with biopsy specimens revealed *H. pylori* infection in 59 of the 90 patients (66%) that were investigated; all of those were associated with chronic gastritis. All of the 31 other patients were negative by both "gold standard" tests (histology and rapid urease test). Serum samples from the 90 patients were analyzed by the IgA and IgG immunoblot assays; IgA antibodies could be detected in 17 of the 59 *H. pylori*-positive patients, resulting in a sensitivity of only 29%. In contrast, the sensitivity of detecting IgG antibodies proved to be 98%, and the IgG immunoblot assay failed to detect *H. pylori* infection in only one patient. The specificities of the IgA and IgG immunoblot assays were demonstrated to be 77 and 71%, respectively. False-positive results by the IgG immunoblot assay were demonstrated in nine patients, with one of them having antibodies against *Yersinia enterocolitica*, as revealed in an immunoblot assay (11). Antibodies against other gastrointestinal pathogens such as *Salmonella* spp., *Campylobacter jejuni*, or *Y. enterocolitica*, as determined by the WIDAL agglutination test, immunoblotting, and enzyme-linked immunosorbent assay (ELISA), respectively, were not found in any of the other patients analyzed in this study. The IgA immunoblot assays were false positive for seven patients. Since the positive and negative predictive values of the IgG immunoblot assay were 87 and 96%, respectively, the IgG antibody class was shown to be superior to the IgA antibody class, which had positive and negative predictive values of only 71 and 36%, respectively.

Antibodies of the IgG class against LMMPs of *H. pylori* were detected in 54 of 58 patients (93%), including 40 patients (69%) having antibodies against UreaA. In contrast, only 47 of 58 patients (81%) had detectable IgG antibodies against HMMPs, including 30 and 21 patients (52 and 36%, respectively) with IgG antibodies against CagA and VacA, respectively (Fig. 1). Antibodies of the IgA class against LMMPs and HMMPs were identified in 15 (88%) and 3 (12%) of 17 patients, respectively. These included IgA antibodies against CagA, VacA, and UreaA that were found in 2, 2, and 15 of 17 patients, respectively (12, 12, and 88%, respectively). Nearly all patients in whom antibodies against LMMPs or HMMPs were detectable were also found to have antibodies against MMMPs, which are supposed to be cross-reactive with antigens of other bacterial species (3, 14, 21).

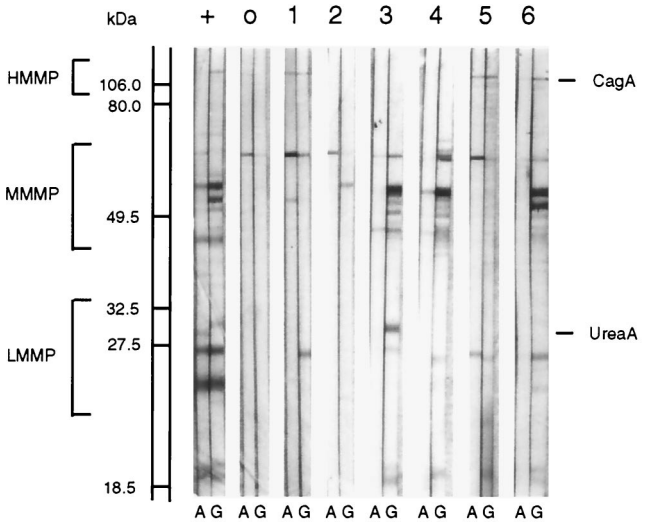

FIG. 1. Representative examples of the results obtained in the IgA (lanes A) and IgG (lanes G) immunoblot assays with human serum samples (lanes 1 to 6). Samples from patients 1, 3, 5, and 6, in lanes 1, 3, 5, and 6, respectively, are considered IgG positive, and samples from patients 1 and 5, in lanes 1 and 5, respectively, are considered IgA positive. The positive and negative controls consisted of serum samples from a known *H. pylori*-infected patient (lane +) or noninfected patient (lane 0), respectively.

DISCUSSION

Several invasive and noninvasive tests have been proven to be accurate for detecting *H. pylori* infection. The current gold standard tests in clinical practice, endoscopy for histology and the rapid urease test, are limited by their relatively high costs and the need for invasive procedures (5, 7, 20, 29).

Determination of antibodies against *H. pylori* presents a relatively simple diagnostic method, with kits that can be used to perform this method now being readily available from commercial sources (5). Several serological assays have been used to detect *H. pylori*-specific antibodies, including ELISA and the agglutination, complement fixation, or indirect immunofluorescence test (1, 6, 20). Methods for detecting both IgG and IgA antibodies exist, although determination of IgG antibodies is felt to be more accurate. The reported sensitivity and specificity of IgG-specific serological tests for *H. pylori* are highly variable, ranging from 30 to 100% (8).

The advantage of using the immunoblot technique in serological assays is its accurate detection of antibodies that react with defined antigens of an infectious agent. Therefore, the immunoblot technique has also been proposed to be useful in serological tests for *H. pylori*. We investigated by the immunoblot assay the *H. pylori*-specific antibody responses of the IgA and IgG classes in serum samples from patients with chronic gastritis. Sera from *H. pylori*-infected patients showed strong reactivities with antibodies against three major protein fractions with low, medium, and high molecular masses. Sera were considered to be positive for *H. pylori*-specific reactivity when antibodies were detected against at least one protein of high molecular mass or at least two proteins of low molecular mass, or both (21). Antibodies against MMMPs were considered to be cross-reactive with antigens of other bacterial species (3, 14, 21).

Since the rapid urease test has been shown to have a sensitivity of 90 to 93% (19), we classified those patients who were positive by both histology and the rapid urease test to be infected with *H. pylori*. In our study, 59 of 90 patients with chronic gastritis were *H. pylori* positive by these gold standard assays. IgG antibodies could be detected in 58 of the 59 *H. pylori*-infected patients. Only one patient was seronegative for *H. pylori*-specific antibodies by the IgG immunoblot assay. This patient was also seronegative by using a CagA-specific ELISA, indicating the possibility that he might have been infected with a CagA-negative *H. pylori* strain. Thus, the sensitivity and specificity of the IgG immunoblot were 98 and 71%, respectively. The performance of the IgA assay was not as convincing as that

of the IgG assay, since its sensitivity and specificity were only 29 and 77%, respectively.

The immunoblot assay for the detection of *H. pylori*-specific IgA and IgG antibodies has so far been used by only a few other investigators. By using histology with gastric biopsy specimens and culture of histology-positive samples, the sensitivity and specificity of the IgG immunoblot have been shown to be approximately 97 and 85%, respectively (3, 21). The diversity of *H. pylori* strains (13, 30) might be responsible for the fact that some patients did not develop antibodies against all antigens of known diagnostic significance. As an example, a patient who was infected with a CagA-positive, VacA-negative strain is expected not to have antibodies against VacA. However, such an infection should be diagnosed by our immunoblot assay, since a serum sample is considered positive by the immunoblot assay if antibodies against at least one HMMP or against at least two LMMPs are detected.

An advantage of our study was also that the results of the two gold standard methods, histology and determination of urease from biopsy specimens, were available for all patients. Whereas the sensitivity of our immunoblot assay was similar to the sensitivities obtained by the other investigators, our immunoblot assay had a lower specificity. This could be due to the fact that the prevalence of *H. pylori*-specific antibodies in a significant number of healthy people must be taken into account. In an analysis of healthy blood donors from the same geographical area as our patient population by the immunoblot technique, it could be shown that 29% had IgG antibodies against *H. pylori* (26a), which is in good accordance with the specificity reported in this study. Therefore, although useful as an additional serological test in a clinical microbiology laboratory, the results of the *H. pylori* IgG immunoblot assay should be interpreted with care, and the factors used to diagnose *H. pylori*-associated disease should always include the clinical presentation of the individual patient. Determination of IgA antibody titers seems to be less useful than analysis of the IgG antibody response for the diagnosis of *H. pylori* infection (20). The detection of IgG antibodies against CagA, VacA, and UreaA in our study confirmed the data that were presented by Andersen and Espersen (3).

The immunoblot assay has proven to be a sensitive test for the diagnosis of *H. pylori* infection. In comparison with other serological tests, this assay seems to be useful for the determination of the true results for specimens with uncertain results that were obtained in these other tests, for example, ELISA (21). In addition, by using the immunoblot assay, antibodies against antigens with different molecular masses (VacA, CagA, and UreaA) can be detected (3).

In conclusion, in a search for noninvasive methods for the diagnosis of *H. pylori* infection, serological tests have received increasing interest. Whereas the sensitivity of the IgA immunoblot assay was shown to be unsatisfactory, this study proved the high degree of sensitivity of the IgG immunoblot assay, whose diagnostic value is its ability to provide a rapid and simple means of diagnosing *H. pylori* carrier status.

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