JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1997, p. 427–432 Vol. 35, No. 2
0095-1137/97/$04.00+0

Immunoblot Assay for Serodiagnosis of *Helicobacter pylori* Infections

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Received 30 July 1996/Returned for modification 19 September 1996/Accepted 12 November 1996

An immunoblot assay for the serological diagnosis of *Helicobacter pylori* infection was evaluated. Serum samples from patients whose gastric biopsy specimens were known to be positive or negative for *H. pylori* on culture were used to establish interpretive criteria for the immunoblot assay. A panel of sera from patients with diseases other than *H. pylori* infection and sera from healthy blood donors were included to validate these criteria. All sera were initially assessed in an enzyme immunoassay (Ge-EIA), based on acid glycine-extracted cell surface proteins of *H. pylori* NCTC 11637. The same antigen extract was used in the immunoblot assay. In addition, the Ge-EIA and the immunoblot assay were compared with a commercially available EIA (Seradyn, Color Vue Pylori). Bands of 110/120 kDa and/or two of five low-molecular-mass proteins (26, 29, 30, 31, and 33 kDa, in any combination) showed a strong correlation with the *H. pylori* culture-positive patients (97.5%) compared to the correlation obtained with the EIA results (Ge-EIA, 87.5%; Seradyn EIA, 92.5%), and the antibody responses to these proteins were considered specific reactions. In 37 of 40 serum samples from culture-negative patients and also in sera from patients with other disorders, a moderate antibody reactivity to the medium-size proteins (43 to 66 kDa) was observed, and these were considered not valuable for a specific immunoblot assay. Among sera from culture-positive patients, 39 of 40 serum samples were defined to be immunoblot positive, and from among sera from culture-negative patients, 3 of 40 serum samples were defined to be immunoblot positive. The use of sera from patients with negative cultures for *H. pylori* as negative controls may decrease the sensitivity due to sampling error and false-negative culture results. Immunoblot assay-positive results were detected among 10% of sera from patients with other diseases, whereas they were detected among 42.5% of sera by the Ge-EIA and 47.5% of sera by the Seradyn-EIA. The higher number of EIA-positive sera in this group reflects a possible cross-reactivity (false-positive EIA result). Of the blood donors, representing asymptomatic but possibly colonized subjects, 24% were immunoblot positive. In conclusion, our data indicate that immunoblotting is more sensitive as well as more specific than EIA. Moreover, it permits detection of antibody responses to specific antigens, e.g., the cytotoxin-associated CagA protein, which may have pathological implications.

Helicobacter pylori infections are usually diagnosed by culture of gastric biopsy specimens obtained by endoscopy and noninvasive methods, such as detection of a serum antibody response or by radiolabeled urea-based breath tests (1, 6, 15). Serological tests have widely been used in epidemiological and posttreatment studies, mostly by enzyme immunoassays (EIAs). These assays are rapid and easy to perform and can be automated. Early serological studies of *H. pylori* used intact bacteria, heated and sonicated cells, and supernatants of ultracentrifuged whole-cell lysates without further modification as solid-phase antigens for EIA. The sensitivities of such tests were remarkably good, but they lacked high specificity due to antigenic cross-reactivity with other bacterial species (5, 9–11, 19, 21, 23). The preparation of acid glycine-extracted cell surface proteins with 0.2 M acid glycine buffer (pH 2.2) (16) was reported to give negligible serological cross-reactivity with *Campylobacter jejuni* (18). On the basis of these findings, this extraction method is now commonly used to produce *H. pylori* antigen for EIA. Still, serological cross-reactivity causes problems with the interpretation of results, as shown in a recent evaluation of eight commercially available EIA kits (8).

By immunoblot assay, antibody reactivity to separate proteins can be analyzed with a higher specificity than by EIA, and false-positive EIA reactions are easily identified. However, until now no consensus regarding the interpretation of immunoblot patterns has been reported.

The aim of the work presented here was to study the acid glycine-released proteins from *H. pylori* NCTC 11637 for use as antigens in an immunoblot assay able to distinguish between specific and nonspecific immunoglobulin G (IgG) antibody responses. We now report on a standardized immunoblot assay for detection of IgG antibodies to *H. pylori* proteins and propose interpretation criteria.

MATERIALS AND METHODS

Patients and controls. A total of 220 serum samples, divided into four groups, were included in the study. Group 1 contained serum samples from patients positive for *H. pylori* on culture of endoscopic biopsy specimens ($n = 40$; mean age, 54 years; age range, 19 to 84 years) and with symptoms of peptic ulcer disease and undefined abdominal pains. This group is the reference panel and is referred to as the theoretical "gold standard." Group 2 consisted of sera from patients with negative for *H. pylori* on culture of endoscopic biopsy specimens ($n = 40$; mean age, 49 years; age range 16 to 91 years) and with symptoms similar to those of the patients whose sera were included in group 1. Group 3 consisted of 40 serum specimens selected to monitor potential cross-reactivity to *H. pylori* components. The serum specimens were from patients suffering from diabetes, cystic fibrosis (with *Pseudomonas aeruginosa* colonization in the lungs), and rheumatoid arthritis (presenting with high rheumatoid factor titers by the Waaler-Rose test), and sera tested positive by *C. jejuni* serology. The sera were selected at the Section of Clinical Microbiology, University Hospital of Lund. Group 4 consisted of serum samples from healthy blood donors ($n = 100$; age range, 18 to 65 years) randomly collected at the Blood Transfusion Unit, University Hospital of Lund and represented sera from an asymptomatic control group. All sera were aliquoted and stored frozen at −20°C until tested.

Biopsy specimens. Of the 80 patients undergoing gastroscopy (group 1 and 2), two to three biopsy specimens from the antrum and corpus were cultured. The specimens were transported to the laboratory in Stuart transport medium, inoculated onto GC Agar Base (GAB)-CAMP plates, and incubated under mi-

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croaerophilic conditions for 3 to 5 days (25). Colonies were identified as *H. pylori* by morphology, urease production, and positive catalase and oxidase reactions.

Bacterial strain and antigen preparation for serology. *H. pylori* NCTC 11637 (CCUG 17874) was used for antigen preparation, which was done by the acid glycine extraction method as described previously (13). Seven batches were prepared and pooled. The protein profile of the pooled extract was compared to that of a single batch by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE).

A partially purified *H. pylori* flagellar preparation (17) was used to monitor cross-reacting antibodies to *H. pylori* flagellin antigens.

Antisera. Polyclonal rabbit antisera were used to identify proteins of *H. pylori* evoking an immune response in rabbits. New Zealand White rabbits were immunized with spiral and coccoid forms of NCTC 11637 and spiral forms of NCTC 11638 and strain 25 (clinical isolate). Each strain and form (spiral and coccoid) were injected into four separate rabbits. The animals were vaccinated subcutaneously (increasing doses from 0.1 to 2 ml) with a suspension of heat-killed cells in phosphate-buffered saline (pH 7.2) (optical density at 595 nm, 1.0) and mixed with equal volumes of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.). Four booster doses were given in Freund's incomplete adjuvant at 10-day intervals.

Five rabbits were immunized with materials obtained by cutting out protein bands separated in an SDS-PAGE gradient (5 to 20%), with approximate M_rs of 31, 60, 62, and 64 kDa (from NCTC 11637) and 120 kDa (from G33) (29) in relation to the molecular size markers. Each protein (10 to 20 μg homogenized in phosphate-buffered saline) was injected subcutaneously. Three booster doses were given, and no adjuvant was used.

In addition, a monoclonal antibody directed against a heat shock protein (Hsp) from *H. pylori* 8826 with an M_r of 62 kDa (Hsp62; kindly supplied by L. Engstrand, Uppsala, Sweden) was included.

EIAs. All sera were initially tested for IgG antibodies to *H. pylori* in the Ge-EIA as described earlier (14), with minor modifications: Maxisorp Immunoplates (Nunc, Roskilde, Denmark) were exchanged for the EIA plates, and as second antibody, an alkaline phosphatase-conjugated anti-human IgG antibody (ORION Diagnostica, Espoo, Finland) was used. The EIA results are expressed as corrected mean absorbance values as a percentage of the value for a reference standard (human gamma globulin; KABI/Pharmacia, Stockholm, Sweden). The cutoff value for seropositivity was set to a relative antibody activity of >35, and relative antibody activity values of between 25 and 35 were regarded as low-positive (borderline) results (13).

Color Vue Pylori-EIA (Seradyn Clinical Diagnostics, Indianapolis, Ind.) is a solid-phase EIA used for the qualitative measurement of IgG antibodies in serum. The antigens used for coating are described as specific antigenic components of the *H. pylori* organism, and the EIA was performed according to the recommendations of the manufacturer. Positive and negative controls were supplemented, and the cutoff value for a run is defined in terms of the average absorbance of the negative controls plus 0.206 optical density units. The interpretation guidelines provided by the manufacturer were followed.

SDS-PAGE and immunoblot assays. SDS-PAGE was performed under reducing conditions (12) by using a Protean II Cell Vertical Electrophoresis equipment (Bio-Rad, Richmond, Calif.). Proteins were separated in a gradient gel (5 to 20%) and a 10% gel, both with a 5% stacking gel. The antigen (125 μg/gel) was diluted in sample buffer (0.5 M Tris-HCl [pH 6.8], 0.5% bromphenol blue, 8% glycerol, 4% SDS, 4% 2-mercaptoethanol), and the mixture was heated at 95°C for 3 min. After cooling, the proteins were loaded onto the gel and were separated for 16 h at 80 V. Molecular size standards (Promega, Scandinavian Diagnostic Services, Falkenberg, Sweden) that included proteins ranging from 14.3 to 97.4 kDa were treated similarly.

The proteins were transferred electrophoretically to an Immobilon polyvinylidene difluoride membrane (pore size, 0.45 μm; Millipore Intertech, Bedford, Mass.) in semidry electroblotter equipment (Ancos, Vig, Denmark) for 1 h at a constant current of 0.8 mA/cm^2. The membrane was saturated by incubation twice for 15 min each time in blocking buffers I and II. Saturated membranes were rinsed once for 10 min in washing buffer and were cut into strips (blocking and washing buffers were from M. Rucheton, Orstom Laboratories, Montpellier, France) (22). The strips were incubated in sera, diluted 1/100 in washing buffer, under gentle agitation for 16 h at 4°C. The strips were then rinsed three times for 5 min each time and were incubated for 2 h at 4°C with horseradish peroxidase-labelled anti-human IgG antibodies or, where needed, anti-rabbit and anti-mouse IgG antibodies (Dako A/S, Glostrup, Denmark) diluted 1/600. After repeated rinsing, membrane-bound antibodies were detected by a reaction in 50 mM sodium acetate buffer containing 0.04% 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, Mo.) and 0.015% H_2O_2. The intensity obtained with the patient sera was compared to that obtained with a strip incubated with human gamma globulin (KABI/Pharmacia), which was included in each analysis as a calibrator. Weakly stained bands were ignored and were considered nonspecific binding (background).

Statistical analyses. The significance of the stained bands formed on immunoblots to major cell surface proteins of *H. pylori* for culture-positive and blood donor sera was evaluated by Fisher's exact test.

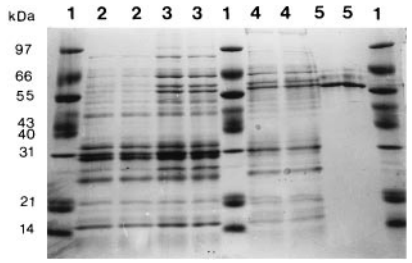

FIG. 1. Protein profiles of acid glycine-extracted cell surface proteins of two *H. pylori* strains and the purified *H. pylori* flagellin. Proteins were separated on an SDS-PAGE gradient (5 to 20%) and stained with Coomassie brilliant blue R-250. Lane 1, molecular size markers; lane 2, a single antigen batch of NCTC 11637; lane 3, pooled antigen batches of NCTC 11637; lane 4, acid glycine-extracted cell surface proteins of strain G33; lane 5, the flagellar preparation, (approximate M_r, 57 kDa).

RESULTS

***H. pylori* antigen.** The acid glycine treatment of *H. pylori* cells is a mild method for releasing surface proteins and minimizes cell lysis. No DNA was detected when the extract was subjected to agarose gel electrophoresis and subsequently stained with ethidium bromide. The pooled *H. pylori* cell surface extracts and the purified flagellin were run in parallell in an SDS-PAGE gradient and were stained with Coomassie brilliant blue R-250. Proteins were identified by their M_rs in relation to the molecular size markers. A protein profile of the antigen extract is shown in Fig. 1. Thirteen prominent protein bands (15, 22, 25, 27, 29, 30, 33, 43, 52, 57, 60, 66, and 87 kDa) and six weaker protein bands (in the range of 17 to 110/120 kDa) were stained (Fig. 1, lane 3). A single batch of the antigen shows the profile for *H. pylori* G33 (Fig. 1, lane 4). In the flagellin preparation one prominent protein of approximately 57 kDa and two minor proteins (62 and 64 kDa) were recorded (Fig. 1, lane 5). The specificities of the *H. pylori* flagellin antigens were evaluated in an immunoblot assay with a *Treponema pallidum*-positive serum sample and another serum sample obtained from a patient with clinically verified Lyme borreliosis. In addition, one strip was incubated with human gamma globulin (KABI/Pharmacia). The sera showed strong antibody reactivity in the 57-kDa region in all three cases (Fig. 2, lanes 1 to 3).

Rabbit antisera and the monoclonal antibody. The immunoblot patterns obtained with the four antisera raised in rabbits with whole cell (spiral and coccoid forms) demonstrated similar reactivities with proteins ranging in size from 14 to 120 kDa (Fig. 3, lanes 1 to 4). The reactivities of rabbit antisera raised against the proteins of 31, 60, 62, 64, and 120 kDa and monoclonal antibody Hsp62 were assessed in an immunoblot assay. The sera reacted strongly to the corresponding proteins, but they also showed reactivity to a few other bands, as demonstrated in Fig. 3 (lanes 5 to 11; lane 10 contained the preimmune serum).

EIAs. In the Ge-EIA, 87.5% of the sera from culture-positive patients (group 1) were positive, and in the Color Vue Pylori-EIA, 92.5% were positive/low positive. None of the sera from culture-negative patients (group 2) were positive, but three serum samples gave low-positive values in the Ge-EIA, and five positive/low-positive results were detected by Color Vue Pylori-EIA. It should be stressed that these were not the same sera that reacted in the two EIAs. For group 3 (potentially cross-reactive sera), 42.5% of sera were positive by the Ge-EIA and 47.5% were positive by the Color Vue Pylori-EIA. For group 4 (blood donors), 29% of the sera were positive by

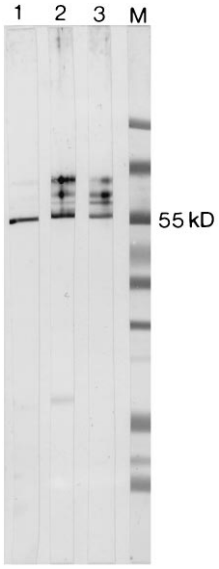

FIG. 2. Cross-reactivity to the *H. pylori* flagellin was evaluated in an immunoblot assay with sera from patients infected with flagellated bacteria other than *H. pylori*. Lane 1, *T. pallidum*-positive serum; lane 2, *B. burgdorferi*-positive serum; lane 3, human gamma globulin (KABI/Pharmacia); lane M, molecular size markers. In all three cases the flagellar proteins were recognized.

the Ge-EIA and 32% were positive by the Color Vue Pylori-EIA (Table 1).

Immunoblot assay. All sera were analyzed in an immunoblot assay with glycine-extracted antigens separated by SDS-PAGE (10% polyacrylamide gel). Representative immunoblot patterns obtained with sera from the different groups are presented in Fig. 4A to D. For group 1, a strong antibody reactivity to three main clusters of *H. pylori* cell surface proteins was observed in sera from culture-positive patients: to proteins with high M_rs (87 to 110/120 kDa), to proteins with medium M_rs (43 to 66 kDa), and to proteins with lower M_rs (26 to 33 kDa) (Fig. 4A; set 1 represents sera with high antibody levels by EIA, set 2 represents sera with moderate antibody levels,

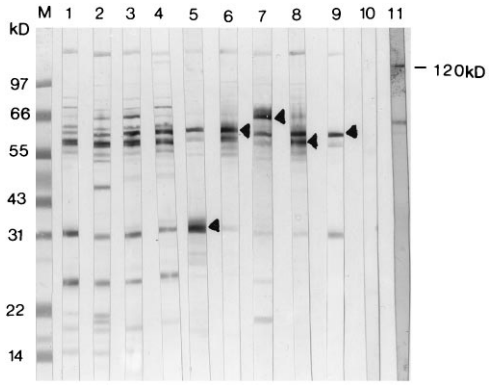

FIG. 3. Rabbit antisera were analyzed by immunoblot assay. Lane 1, rabbit antiserum to whole cells of *H. pylori* NCTC 11637; lane 2, antiserum to whole cells of strain NCTC 11638; lane 3, antiserum to whole cells of strain 25; lane 4, rabbit antiserum to coccoid forms of strain NCTC 11637; lane 5, rabbit antiserum to the 31-kDa protein; lane 6, rabbit antiserum to the 62-kDa protein; lane 7, rabbit antiserum to the 64-kDa; lane 8, rabbit antiserum to the 60-kDa protein; lane 9, monoclonal antibody to the Hsp62 protein; lane 10, preimmune rabbit serum; lane 11, rabbit antiserum to the 120-kDa protein; lane M, molecular size markers (indicated in on the left, in kilodaltons).

TABLE 1. Outcomes of two EIAs and immunoblot assay

Group and result	No. of samples			No. of samples positive by an EIA/no. positive by immunoblot assay	
	Ge-EIA	Seradyn HP-EIA[a]	Immunoblot assay	Ge-EIA/IB	Seradyn HP-EIA/IB
Group 1[b]					
Positive	31	34	39	35[f]/39	37[f]/39
Low positive	4	3			
Negative	5	3	1		
Group 2[c]					
Positive	0	4	3	3[g]/3	5[g]/3
Low positive	3	1			
Negative	37	35	37		
Group 3[d]					
Positive	13	15	4	17[f]/4	19[f]/4
Low positive	4	4			
Negative	23	21	36		
Group 4[e]					
Positive	26	21	24	29[f]/24	32[f]/24
Low positive	3	11			
Negative	71	68	76		

[a] Color Vue Pylori EIA.
[b] Culture-positive patient sera ($n = 40$).
[c] Culture-negative patient sera ($n = 40$).
[d] Sera from patients with diseases other than *H. pylori* infection ($n = 40$).
[e] Blood donor sera ($n = 100$).
[f] Data for low-positive sera are included among the data for positive sera.
[g] EIA negative.

and set 3 represents sera with low or negative antibody levels). In four serum samples reactivity to the 110/120-kDa protein complex was not detected, but these sera presented strong antibody responses to the low-M_r proteins (Fig. 4A, lanes 8, 9, 20, and 27). One serum sample (Fig. 4A, lane 30) showed no reactivity to either high- or low-M_r proteins. The single negative serum sample from culture-positive patients was negative by EIA as well. Possibly, the serum sample was drawn too early during the course of disease. For sera from culture-negative patients in group 2, most sera reacted with medium-size proteins (Fig. 4B, lanes 1 to 5), but three serum samples negative by EIA stained two of the low-M_r proteins (Fig. 4B, lanes 6 to 8). Of the three serum samples, two were from patients with nonulcer dyspepsia and one was from a patient with relapsing ulcer disease. For the sera from the diabetic patients in group 3, all of which were negative by EIA, a weak immune response to medium-size proteins was noted. Sera from cystic fibrosis patients (infected with *P. aeruginosa*), patients with rheumatoid arthritis, and *C. jejuni*-infected individuals showed strong to moderate reactivities to the medium-size proteins (43 to 66 kDa) (Fig. 4C). Four of 40 serum samples from patients in group 3 showed antibody responses to two of the low-M_r proteins (data not shown). None of the sera in this group recognized the 110/120-kDa protein complex. Among the blood donors (group 4), 24 of 100 serum samples showed antibody reactivity to the 110/120-kDa complex and/or to two of the low-M_r proteins (26 to 33 kDa) (Fig. 4D).

The IgG antibody reactivities to major cell surface antigens of *H. pylori* in culture-positive patient sera and blood donors were compared. Significant values were obtained for 11 proteins of the following sizes: 110/120, 87, 66, 64, 60, 57, 52, 47, 43, 31/33, and 26/29 kDa ($P < 0.001$; two-tailed).

Since medium-size proteins (43 to 66 kDa; e.g., subunits of

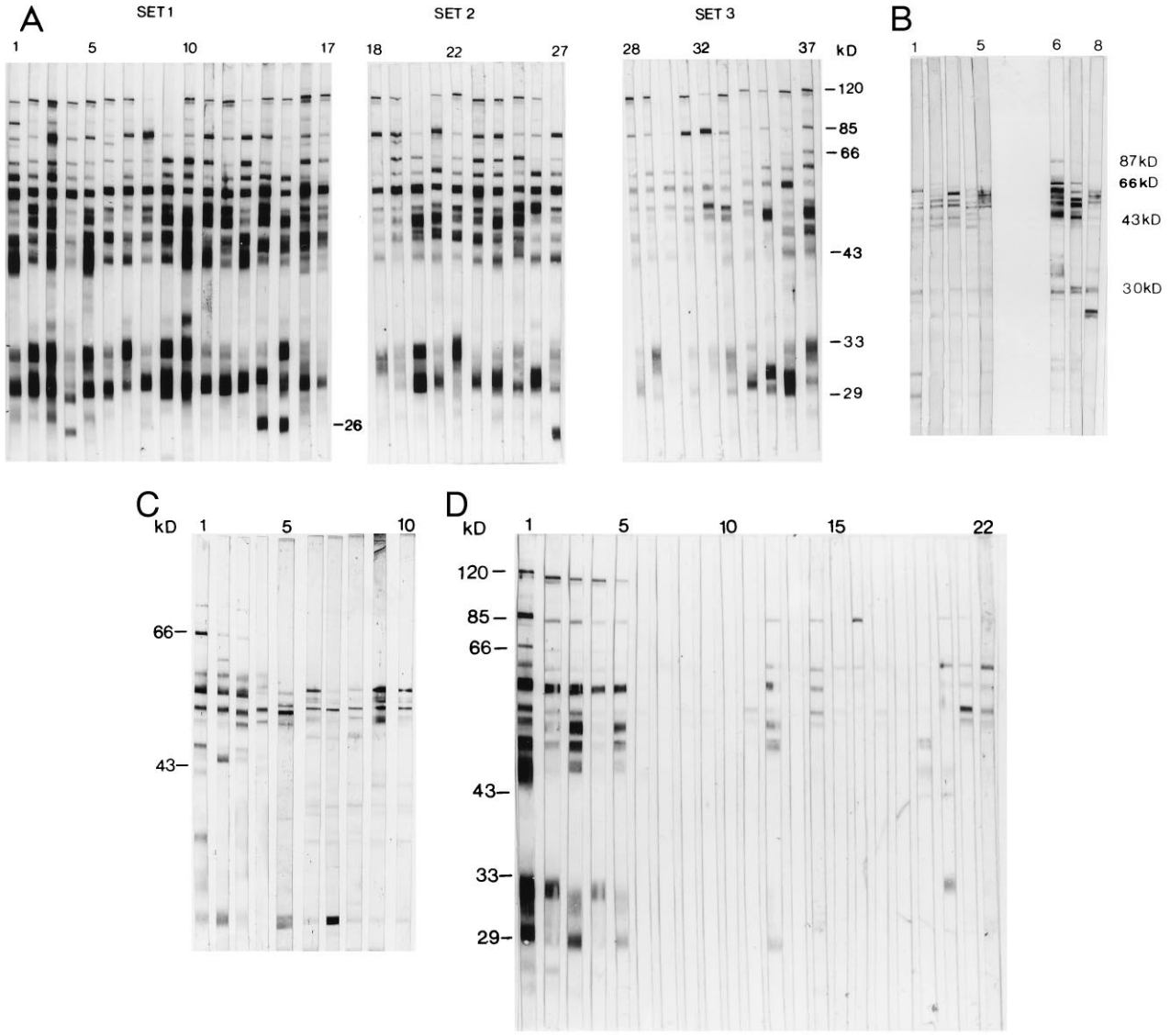

FIG. 4. (A) Representative immunoblot results with sera from group 1 (culture-positive patient sera). Set 1, stained bands in sera with high antibody levels by EIA; set 2, stained bands in sera with moderate antibody levels by EIA; set 3, stained bands in sera with low or negative antibody levels by EIA. (B) Immunoblot analysis of sera from group 2 (culture-negative patients). Lanes 1 to 5, negative results; lanes 6 to 8, three serum samples that were defined to be immunoblot positive. (C) Representative immunoblot results for sera from group 3 (potentially cross-reactive sera). Lanes 1 to 5, *P. aeruginosa*-infected patient sera; lanes 6 to 10, sera from patients with rheumatoid arthritis reacting with the medium-size proteins (43 to 66 kDa). (D) Representative immunoblot results for sera from group 4 (healthy blood donors). Lanes 1 to 5, EIA- and immunoblot assay-positive blood donor sera; lanes 6 to 22, EIA- and immunoblot assay-negative blood donor sera.

urease, Hsp's, and flagellins) showed cross-reactivity with sera from patients in groups 2 and 3, antibody reactivity in this region was not estimated. On the basis of these findings, the criteria for a positive immunoblot result was defined as follows. Sera staining with a high-M_r protein (87, 94, and 110/120 kDa) or at least two of five of the low-molecular-mass proteins (26, 29, 30, 31, and 33 kDa), or combinations of both, were scored as positive. Results of the EIA and the immunoblot assay are compared and presented in Table 1.

DISCUSSION

H. pylori possesses a number of antigens inducing an immune response in infected persons (3, 10, 14, 28), and detection of antibodies to *H. pylori* by IgG EIA provides the basis for serology. Recently, Cutler et al. (6) concluded that the noninvasive tests (EIA, PCR, and urea-based breath tests) are as accurate as the invasive tests (e.g., culture of gastric biopsy or histologic examination) at predicting the *H. pylori* infection status of untreated patients. Sobala et al. (24) suggested that a strategy of preendoscopic screening based on information regarding patient age (younger than 45 years), use of nonsteroidal anti-inflammatory drugs, and *H. pylori* antibody status may allow those patients for whom endoscopy is indicated to be clearly defined. In fact, such an approach may reduce the endoscopy workload by roughly 23%, thereby resulting in a considerable reduction in costs.

This study was designed to identify immunogenic *H. pylori* cell surface proteins especially valuable in *H. pylori* serology and to evaluate a two-test approach (EIA and immunoblot assay) with high sensitivity and specificity. The antigen used in such tests should preferably have a number of species-specific

epitopes to give a high test sensitivity and reduce unspecific reactions with other species. SDS-PAGE analysis of surface proteins from strain NCTC 11637 and another *H. pylori* strain (strain G33) gave similar protein profiles by SDS-PAGE (Fig. 1 lane 4), as well as an almost identical outcome by EIA and immunoblot assay (data not shown). Most of the proteins in the pooled batch showed a higher intensity by SDS-PAGE than a single batch of the extract (Fig. 1, lane 2). This might be due to mixing of several batches, which gives a more even amount of individual proteins, which in turn gives a product of higher quality and uniformity. Another advantage of pooling several antigen batches is that batch-to-batch variations are avoided. It is important to include the 110/120-kDa protein complex in antigen mixtures since they have been identified as highly species-specific antigen markers for cytotoxin-producing strains (26, 29). Of the low-M_r proteins, a 30-kDa protein, also represented among the antigens detected in our study, has recently been described as being *H. pylori* specific (4). Of the culture-positive patient sera, 93% showed strong antibody responses to proteins between 29 and 33 kDa, as has also been reported by Andersen et al. (2). For sera from culture-negative subjects, a very faint staining of the 29/30-kDa protein was seen (55%); this staining might have been due to cross-reactivity to other urease-producing bacteria (27). Thus, for patients infected with a noncytotoxin-producing strain lacking antibodies to the 110/120-kDa protein complex, the low-M_r proteins could be included for a discriminative immunoblot assay. In a previous study by O'Toole et al. (20), another major-low-molecular-mass (26-kDa) cell surface protein was shown to be antigenically unique to *H. pylori*. Reactivity to this protein was not frequently seen with immunoblot-positive sera in our evaluation. However, another method for antigen preparation was used (octyl glucoside extraction), which probably altered the protein content.

Three serum samples from culture-negative patients were defined as immunoblot positive. One patient suffered from relapsing ulcer disease and two suffered from nonulcer dyspepsia. Possible explanations for the negative biopsy cultures could be that *H. pylori* was not recovered from the biopsy specimens or that the patients had residual seropositivity after treatment for a *Helicobacter* infection. However, the use of sera from culture-negative patients as true negative controls is difficult due to sampling error and transport problems. Conversely, one patient in this study with documented *Helicobacter* infection failed to seroconvert.

Cross-reactivity was monitored with a panel of sera from patients with infections that usually show serological cross-reactivity. Between 42 and 47% of these serum samples were positive by the two EIAs, compared to 10% confirmed to be positive by the immunoblot assay. False-positive EIA results could thus be detected and the samples could be defined as negative. Cross-reactivity was also seen with two serum samples from patients infected with flagellated bacteria other than *H. pylori* (*T. pallidum* and *Borrelia burgdorferi*). Moreover, all sera from *P. aeruginosa*-infected cystic fibrosis patients recognized Hsp's in the *H. pylori* immunoblot. The immunoblot assay findings that the medium-size proteins (43 to 66 kDa) are responsible for cross-reactivity with antigens of other bacterial species are in agreement with data presented by Bazillou and coworkers (3). The specificity of high- and low-M_r proteins in the serodiagnosis of *H. pylori* infections has been confirmed in our study as well.

Good agreement was found between positive culture results and immunoblot-positive sera (97.5%), compared to that found between culture results and EIA results (87.5 to 92.5%). Thus, the immunoblot appears to be a more sensitive assay, especially with sera with low levels of antibodies that were not detected by EIA. Immunoblotting allows for the analysis of the immune response to a number of defined antigens which EIA does not and for the differentiation of species-specific and cross-reacting antibodies. It can be used as an additional indicator of antibody response when the outcome of EIA is doubtful in the preendoscopic screening of dyspeptic patients or when is not possible to culture gastric biopsy specimens, e.g., with children. Densitometric scanning of the intensity of each band instead of a subjective visual recording could be an advantage for further improvement of the specificity and accuracy of the assay (7). The sensitivity and specificity of the EIA and the immunoblot assay will be increased if the proteins with high and low molecular masses are isolated and purified to obtain a more defined protein mixture.

ACKNOWLEDGMENTS

We thank N. Figura for providing *H. pylori* G33 and C. Penn for the flagellin preparation. We are grateful to B. Zeeberg for excellent technical assistance and I. Frick for photography. We also thank Seradyn Clinical Diagnostics for supplying the diagnostic kit.

This study was supported by grants from the Swedish Medical Research Council (16x-4723); The Crafoord Foundation; M. Bergvalls Foundation; LFTP Foundation; and Medical Faculty, University of Lund.

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