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Cross-Reactive Antigens Shared by *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Campylobacter jejuni*, and *Haemophilus influenzae* May Cause False-Positive Titers of Antibody to *H. pylori*

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Cystic fibrosis (CF) patients suffer from many of the gastrointestinal conditions which occur in non-CF individuals, e.g., dyspepsia and peptic ulceration. These symptoms may be caused by *Helicobacter pylori* but could also be due to either pancreatic insufficiency or the intensive antibiotic treatment used in CF patients. Since CF patients chronically infected with *Pseudomonas aeruginosa* produce antibodies against a wide range of antigens, including antigens common to many other bacteria, e.g., GroEL and lipopolysaccharide, we studied, by the Western blot (immunoblot) technique, the specificity of immunoglobulin G antibodies to *H. pylori* in Danish CF patients chronically infected with *P. aeruginosa*, CF patients without *P. aeruginosa* infection but with *Haemophilus influenzae* infection, patients with dyspeptic ulcers associated with *H. pylori*, and patients recovering from acute *Campylobacter jejuni* or *Campylobacter coli* infection. Sera from CF patients with chronic *P. aeruginosa* or *H. influenzae* infection and patients recovering from acute *C. jejuni* infection cross-reacted with *H. pylori* antigens. A strong cross-reacting protein antigen at approximately 14 kDa and minor cross-reactive antigens at approximately 27, 30, and 60 kDa (the heat shock protein GroEL is equivalent to the common antigen of *P. aeruginosa*) could be demonstrated. The results of this study show that high immunoglobulin G antibody titers against *H. pylori* in CF patients cannot be regarded as indicating present or past *H. pylori* infection unless their specificity is proven by absorption studies.

Dyspepsia is frequently observed in patients with cystic fibrosis (CF) (22). Radiographic studies of the duodenum in CF patients with abdominal pain have revealed abnormalities such as thickened mucosal folds, effacement of normal folds, and peptic ulcerations in approximately 85% of the investigated patients (35, 43). The epigastric pain might be caused by the intensive antibiotic treatment used in these patients or pancreatic insufficiency. The abdominal symptoms in CF patients could also be due to infection with *Helicobacter pylori* since this bacterium has been established as the most common cause of chronic gastritis (29, 37) and peptic ulcer (46).

The immunoglobulin G (IgG) antibody level to *H. pylori* increases in 10 to 15% of normal asymptomatic children with increasing age (3). The prevalence of antibodies to *H. pylori* in patients with CF was compared with that in non-CF controls by Przyklenk et al. (36), who found that the seroprevalence of *H. pylori* was the same for both groups (36). In contrast to this, Littlewood (28) suggested an increased prevalence of *H. pylori* in CF patients and proposed that this infection should be excluded in patients with persisting upper gastrointestinal symptoms.

H. pylori has been suspected to cross-react antigenically with other gram-negative bacteria (19). The cross-reactions of the flagellar antigen with *Campylobacter jejuni* are well established (30, 33). The common protein antigen of gram-negative bacteria (the heat shock protein GroEL of ~60 kDa is equivalent to the common antigen of *Pseudomonas aeruginosa*) (19, 41) and lipopolysaccharide (LPS) may be other potential candidates (1, 2, 7, 34). It is well-known that CF patients chronically infected with *P. aeruginosa* produce antibodies to a variety of corresponding antigens (10, 16, 26) and LPS (12, 13, 17), including cross-reactive antigens such as the common protein antigen of *P. aeruginosa*, GroEL (19).

The purpose of this study was to investigate and, if possible, to identify the cross-reacting antigens in *H. pylori*, *P. aeruginosa*, *Haemophilus influenzae*, and *C. jejuni* by using sera from CF patients with chronic *P. aeruginosa* infection with or without additional *H. influenzae* infection and sera from patients with *H. pylori* or *C. jejuni* infection. The IgG antibody responses to the four bacteria were analyzed by the Western blot (immunoblot) technique. Cross-reactions were identified by the presence of antibodies to *P. aeruginosa* GroEL.

MATERIALS AND METHODS

***H. pylori*-positive serum pool.** Serum samples from seven patients with dyspeptic symptoms and peptic ulcer were pooled in equal amounts. The diagnosis was established by either growth of *H. pylori* from gastric mucosal biopsy specimens (5) or microscopy showing the presence of *Helicobacter*-like organisms in histological sections of formalin-fixed biopsy specimens stained with hematoxylin and eosin or by immunohistochemical staining (4). These sera contained antibodies against all detectable proteins of *H. pylori* in the Western blot.

***P. aeruginosa*-positive serum pool.** Serum samples from 10 CF patients with chronic *P. aeruginosa* lung infections, defined by the continuous presence of the bacteria in the sputum for 6 months and/or an antibody response of two precipitins or more (21), were pooled in equal amounts. Diagnosis of CF was established on the basis of abnormal sweat electrolytes and respiratory and gastrointestinal symptoms, including meconium ileus. The CF patients are seen every month in the outpatient clinic at Rigshospitalet (32) for medical examination, pulmonary function tests by spirometry (Dräger Werk AG, Lübeck, Germany), microscopy and culture of sputum, and antibody response to *P. aerugi-*

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nosa (21). The lung infections are treated aggressively as reported previously (20, 32, 45).

***C. jejuni*- or *Campylobacter coli*-positive serum pool.** Serum samples from five patients with acute diarrhea, positive stool cultures for *C. jejuni* or *C. coli*, and high levels of IgG against *C. jejuni* measured by enzyme-linked immunosorbent assay (ELISA) were pooled in equal amounts.

***H. influenzae*-positive serum pool.** Serum samples from three CF patients with *H. influenzae* lung infections, defined by the presence of more than two precipitins against *H. influenzae* examined by crossed immunoelectrophoresis as detailed previously (18), and no history of *P. aeruginosa* infection were pooled in equal amounts.

All serum pools were stored separately at −20°C until use.

Antisera specific to *P. aeruginosa* GroEL. The following antibodies were used: (i) polyclonal rabbit antibodies against *P. aeruginosa* GroEL, (ii) mouse monoclonal antibodies (C2-F15) specific for *P. aeruginosa* GroEL, and (iii) mouse monoclonal antibodies (C2-F12) against a conserved part of *P. aeruginosa* GroEL also present in GroEL proteins of several other bacteria (24, 42).

Bacterial strains. A clinical isolate of *H. pylori* (CH-20429) from a human gastric mucosal biopsy of an adult patient with a duodenal ulcer was subcultured microaerophilically at 37°C on chocolate agar plates for 40 h. This strain has been used in other studies (2).

P. aeruginosa PAO 579, which stably maintains a mucoid phenotype and is International Antigen Typing System O:2/5 (kindly provided by J. R. W. Govan, Edinburgh, United Kingdom) (14, 15, 25), was cultured on a modified Conradi-Drigalski's substrate for 18 h at 37°C.

Clinical isolates of *C. jejuni* RH-240481 (isolated from a blood culture from a child with leukemia) and *C. coli* C.I.P. 70.80 (the type strain; isolated from feces from an adult with acute diarrhea) were subcultured microaerophilically on 5% blood agar plates for 18 h.

A clinical isolate of *H. influenzae* capsular type b (RH-19648) was subcultured aerobically on blood agar plates for 18 h at 37°C.

Whole-cell preparations. The bacterial cultures were harvested and washed twice in sterile distilled water and centrifuged at 7,000 × *g* for 10 min. Preparations of *C. jejuni* and *C. coli* were mixed in equal amounts. The bacterial pellets were stored at −20°C.

Sonicated cell preparations. Whole-cell preparations of *H. pylori*, *P. aeruginosa*, *C. jejuni*, and *H. influenzae* were harvested in sterile water and resuspended to a concentration of 0.5 g (wet weight) per ml of phosphate-buffered saline (PBS; pH 7.4) when used. The bacteria were broken by sonication at 20,000 Hz for 45 s; this process was performed a total of five times with a Rapidis 300 19-mm probe with a 9.5-mm tip. The preparations were cooled during sonication by immersion in ice water. The sonicated suspensions were stored at −20°C. Antigens from the culture media were not present in the final antigen preparations (whole cells or sonicate) (38, 39).

Purification of *P. aeruginosa* recombinant GroEL. Recombinant 60-kDa GroEL of *P. aeruginosa* was purified as described elsewhere (23). Briefly, two primers covering the coding sequence of the *P. aeruginosa* P1118 *groEL* gene were synthesized. PCR was performed under standard PCR conditions, and the PCR product (~1.7 kbp) was cut with *Nde*I and *Bam*HI and ligated into pET16b vector (Novagen, Madison, Wis.). The ligated material was transformed into competent *Escherichia coli* B121(DE3). The cells were grown in Luria-Bertani broth with carbenicillin for 3 h at 37°C, induced with isopropyl-β-D-thiogalactopyranoside, and sonicated. The culture was centrifuged, and the recombinant protein was purified from the supernatant with a Ni^{2+} charged His-Bind resin column and eluted in 1 M imidazole–0.5 M NaCl–20 mM Tris-HCl (pH 7.9). The 60-kDa GroEL protein was identified with specific monoclonal anti-*P. aeruginosa* GroEL antibodies (23, 24). Protein concentration was measured by the Bio-Rad protein assay (range, 0.2 to 1.4 mg/ml; at an optical density at 595 nm) with bovine serum albumin (Sigma) as the standard. The recombinant GroEL protein was cut with Factor Xa (restriction protease factor Xa; Boehringer GmbH, Mannheim, Germany) and stored at −20°C until use.

Absorption of sera. On the basis of previous results (2), 2 parts of the whole-cell preparations of *H. pylori* CH-20429, *P. aeruginosa* PAO 579, *C. jejuni* RH-200481 or *C. coli* C.I.P. 70.80, and *H. influenzae* RH-19648 (0.5 g/ml) were mixed with 1 part of the ultrasonicated preparations of the same strains (0.5 ml [wet weight]) in PBS (pH 7.4). Equal volumes of each of the antigen suspensions and the positive serum pools diluted 1:25 in PBS (pH 7.4) were mixed in a Vortex mixer for 30 s, incubated at room temperature for 1 h, and remixed for 30 s. The suspensions were incubated overnight at 4°C and centrifuged at 12,000 × *g* for 10 min, and the supernatants were stored at 4°C. The absorptions of the *H. pylori*-, *P. aeruginosa*-, *C. jejuni*-, *C. coli*-, and *H. influenzae*-positive serum pools were repeated with all bacterial strains. The absorbed sera were used in the immunoblotting studies with the reference strain.

SDS-polyacrylamide gel electrophoresis. The whole-cell preparation and the ultrasonicated cell preparation were diluted 1:30 in PBS (pH 7.4) and mixed with equal volumes of sample buffer containing 0.4% sodium dodecyl sulfate (SDS) and 4.8% (wt/vol) DL-dithiothreitol. The suspensions were boiled for 5 min in a water bath.

Electrophoresis was carried out as described by Laemmli (27) with a 15% polyacrylamide separation gel and a 5% polyacrylamide stacking gel. Relative molecular weight was determined by the use of reference proteins (low-molecular-weight kit; Pharmacia Fine Chemicals).

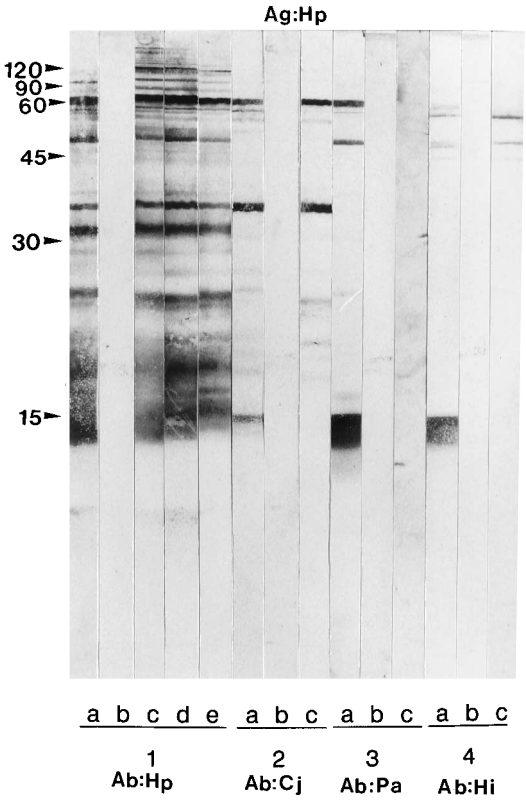

FIG. 1. Western blot analysis showing antibody responses to *H. pylori* antigens. Lanes: 1a, unabsorbed *H. pylori* antibody pool; 1b to 1e, *H. pylori* antibody pool absorbed with *H. pylori* (lane 1b), *P. aeruginosa* (lane 1c), *C. jejuni* (lane 1d), and *H. influenzae* (lane 1e) antigens; 2a, unabsorbed *C. jejuni* antibody pool; 2b and 2c, *C. jejuni* antibody pool absorbed with *H. pylori* (lane 2b) and *C. jejuni* (lane 2c) antigens; 3a, unabsorbed *P. aeruginosa* antibody pool; 3b and 3c, *P. aeruginosa* antibody pool absorbed with *H. pylori* (lane 3b) and *P. aeruginosa* (lane 3c) antigens; 4a, unabsorbed *H. influenzae* antibody pool; 4b and 4c, *H. influenzae* antibody pool absorbed with *H. pylori* (lane 4b) and *H. influenzae* (lane 4c) antigens. Abbreviations: Ag:Hp, *H. pylori* antigens; Ab:Hp, *H. pylori* antiserum; Ab:Cj, *C. jejuni* antiserum; Ab:Pa, *P. aeruginosa* antiserum; Ab:Hi, *H. influenzae* antiserum.

Western blot analysis. Western blot analysis was carried out as described previously (11, 40). Electrophoretic transfer of protein from unstained SDS-polyacrylamide gels was performed by a modification of the technique described by Towbin et al. (44). A nitrocellulose gel was assembled, and the protein was transferred to the nitrocellulose paper (HAWP 2930; pore size, 0.45 μm; Millipore) at 20°C for 18 h at 24 V in 25 mM Tris-hydrochloride–0.192 M glycine (pH 8.4) containing methanol (4.9 M). After transfer, the remaining binding sites on the paper were blocked by incubation with Tween 20 (2% [wt/vol]) for 30 min. The nitrocellulose sheets were then incubated for 1 h at 20°C with human serum, diluted 1:100 in Tris-HCl buffer (pH 7.4) with 2% (wt/vol) Tween 20. The nitrocellulose sheet was then washed three times in Tris-HCl buffer (pH 7.4) with 2% (wt/vol) Tween 20 and 10% NaCl and then incubated for 1 h at 20°C with horseradish peroxidase-conjugated rabbit anti-human IgG antibodies (Tago, Inc., Burlingame, Calif.) diluted 1:2,000 in Tris-HCl buffer (pH 7.4) containing 2% (wt/vol) Tween 20. The washing was repeated as described above, and the sheets were incubated in a citrate-phosphate buffer (pH 5.0) with 5 mM H_2O_2–tetramethylbenzidine (Merck) and dimethyl sulfoxide (Merck) for 10 min at 20°C. The enzyme reaction was stopped by washing the sheets in distilled water.

RESULTS

Antibody response to *H. pylori*. The immunoblot revealed the expected bands (2) when the *H. pylori* pool was tested against the *H. pylori* antigens (Fig. 1, lane 1a). Antibodies to *H. pylori* were completely absorbed from the *H. pylori* pool by the *H. pylori* antigens (lane 1b). No antibodies to *H. pylori* were eliminated when the *H. pylori*-positive antibody pool was ab-

TABLE 1. Major *H. pylori* antigens cross-reactive with *P. aeruginosa*, *C. jejuni*, and *H. influenzae*

Antigen	Cross-reactivity with *H. pylori* antigen[a]			
	14 kDa[b]	46 to 50 kDa	54 to 56 kDa	GroEL
P. aeruginosa	×	×	×	×
C. jejuni	×	−	×	−
H. influenzae	×	−	−	×

[a] Symbols: ×; cross-reactivity detected; −, no cross-reactivity detected.
[b] Molecular size of antigen.

sorbed with *P. aeruginosa*, *C. jejuni*, or *H. influenzae* antigens (lanes 1c, d, and e).

The immunoblot revealed only a few bands when the *C. jejuni* pool was tested against the *H. pylori* antigens (Fig. 1, lane 2a). Complete absorption was found with the *H. pylori* antigens (lane 2b). When the *C. jejuni* pool was absorbed with *C. jejuni* antigens, two bands of approximately 54 and 14 kDa were removed (lane 2c), indicating a cross-reaction (Table 1).

The immunoblot revealed few bands when the *P. aeruginosa* pool was tested against *H. pylori* antigens (Fig. 1, lane 3a). Complete absorption was found with the *H. pylori* antigens (lane 3b). When the *P. aeruginosa* pool was tested against *P. aeruginosa* antigens, complete absorption of bands with molecular sizes of approximately 46 to 50 kDa, two bands of 56 kDa and 61 to 60 kDa, and a 14-kDa band (lane 3c) was revealed, indicating a cross-reaction (Table 1).

The immunoblot revealed few bands when the *H. influenzae* pool was tested against the *H. pylori* antigens (Fig. 1, lane 4a). Complete absorption was found with the *H. pylori* antigens (lane 4b). The *H. influenzae* antigen absorbed two bands of approximately 60 to 61 kDa and 14 kDa (lane 4c), indicating a cross-reaction (Table 1).

Antibody response to *P. aeruginosa*. The immunoblot revealed a great number of bands when the *P. aeruginosa* pool was tested against the *P. aeruginosa* antigens (Fig. 2, lane 1a). Antibodies to *P. aeruginosa* were completely absorbed from the *P. aeruginosa* pool by *P. aeruginosa* antigens (lane 1b). No antibodies to *P. aeruginosa* were eliminated when the *P. aeruginosa* pool was absorbed with the *H. influenzae*, *C. jejuni*, and *H. pylori* antigens (lanes 1c, d, and e).

The immunoblot revealed several bands when the *H. pylori* pool was tested against *P. aeruginosa* antigens (Fig. 2, lane 2a), and complete absorption was found with the *P. aeruginosa* antigens (lane 2b). When the *H. pylori* antibody pool was absorbed with *H. pylori* antigens, three bands were removed, a strong 14-kDa band and two weak bands of 27 kDa and approximately 80 to 90 kDa (lane 2c), indicating a cross-reaction.

The immunoblot revealed only a few bands when the *C. jejuni* pool was tested against *P. aeruginosa* antigens (Fig. 2, lane 3a). Complete absorption was found when the *C. jejuni* pool was absorbed with *P. aeruginosa* antigens (lane 3b). Absorption with *C. jejuni* antigens also revealed complete absorption of all four small-molecular-size bands, of 36, 35, 30, and 14 kDa (lane 3c), indicating a cross-reaction.

The *H. influenzae* pool also revealed a few bands when tested with the *P. aeruginosa* antigens (Fig. 2, lane 4a). Complete absorption was found when the *H. influenzae* pool was absorbed with *P. aeruginosa* antigens (lane 4b), whereas absorption with *H. influenzae* antigens removed only a band of 14 kDa (lane 4c).

Antibody response to *C. jejuni*. When the *C. jejuni* pool was tested against *C. jejuni* antigens, the immunoblot revealed several large-molecular-size bands, whereas only one band below

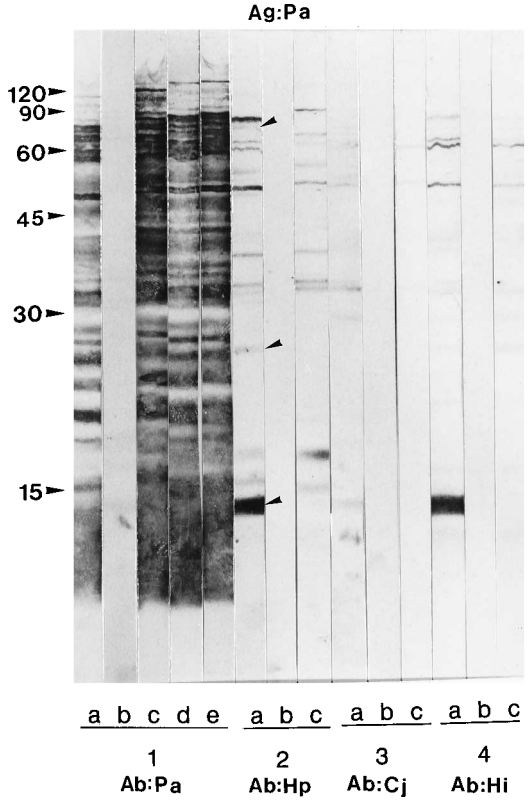

FIG. 2. Western blot analysis showing antibody responses to *P. aeruginosa* antigens. Lanes: 1a, unabsorbed *P. aeruginosa* antibody pool; 1b to 1e, *P. aeruginosa* antibody pool absorbed with *P. aeruginosa* (lane 1b), *H. influenzae* (lane 1c), *C. jejuni* (lane 1d), and *H. pylori* (lane 1e) antigens; 2a, unabsorbed *H. pylori* antibody pool; 2b and 2c, *H. pylori* antibody pool absorbed with *P. aeruginosa* (lane 2b) and *H. pylori* (lane 2c) antigens; 3a, unabsorbed *C. jejuni* antibody pool; 3b and 3c, *C. jejuni* antibody pool absorbed with *P. aeruginosa* (lane 3b) and *C. jejuni* (lane 3c) antigens; 4a, unabsorbed *H. influenzae* antibody pool; 4b and 4c, *H. influenzae* antibody pool absorbed with *P. aeruginosa* (lane 4b) and *H. influenzae* (lane 4c) antigens. Arrowheads indicate 14-, 27-, and 80- to 90-kDa antigens. Abbreviations: Ag:Pa, *P. aeruginosa* antigens; Ab:Pa, *P. aeruginosa* antiserum; Ab:Hp, *H. pylori* antiserum; Ab:Cj, *C. jejuni* antiserum; Ab:Hi, *H. influenzae* antiserum.

30 kDa was revealed. It was remarkable that the 14-kDa band was absent (Fig. 3, lane 1a). A 50-kDa band was only partly absorbed, whereas the remaining bands were completely absorbed when the *C. jejuni* pool was absorbed with the *C. jejuni* antigens (lane 1b). A 22-kDa band was removed when the *C. jejuni* pool was absorbed with *P. aeruginosa* antigens (lane 1c), whereas no antibodies to *C. jejuni* were eliminated when the *C. jejuni* pool was absorbed with *H. pylori* or *H. influenzae* antigens (lanes d and e).

The immunoblot revealed several bands, including the 14-kDa band, when the *H. pylori* pool was tested against the *C. jejuni* antigens (Fig. 3, lane 2a). Complete absorption was found except for the 50-kDa antigen when absorption was with *C. jejuni* antigens (lane 2b). When the *H. pylori* pool was absorbed with *H. pylori* antigens, three bands of approximately 46, 30, and 14 kDa were removed (lane 2c), indicating a cross-reaction.

The immunoblot revealed several bands of about 30 kDa and only a 14-kDa band below 30 kDa when the *P. aeruginosa* pool was tested against *C. jejuni* antigens (Fig. 3, lane 3a). The absorption of the *P. aeruginosa* pool with *C. jejuni* was only partial since at least four bands above 50 kDa were still present but reduced (lane 3b). Only the 14-kDa band was absorbed by

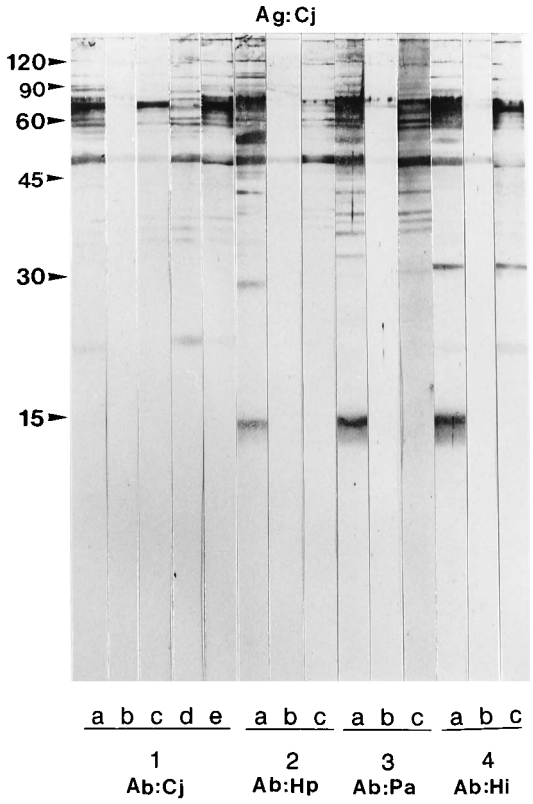

FIG. 3. Western blot analysis showing antibody responses to *C. jejuni* antigens. Lanes: 1a, unabsorbed *C. jejuni* antibody pool; 1b to 1e, *C. jejuni* antibody pool absorbed with *C. jejuni* (lane 1b), *P. aeruginosa* (lane 1c), *H. pylori* (lane 1d), and *H. influenzae* (lane 1e) antigens; 2a, unabsorbed *H. pylori* antibody pool; 2b and 2c, *H. pylori* antibody pool absorbed with *C. jejuni* (lane 2b) and *H. pylori* (lane 2c) antigens; 3a, unabsorbed *P. aeruginosa* antibody pool; 3b and 3c, *P. aeruginosa* antibody pool absorbed with *C. jejuni* (lane 3b) and *P. aeruginosa* (lane 3c) antigens; 4a, unabsorbed *H. influenzae* antibody pool; 4b and 4c, *H. influenzae* antibody pool absorbed with *C. jejuni* (lane 4b) and *H. influenzae* (lane 4c) antigens. Abbreviations: Ag:Cj, *C. jejuni* antigens; Ab:Cj, *C. jejuni* antiserum; Ab:Hp, *H. pylori* antiserum; Ab:Pa, *P. aeruginosa* antiserum; Ab:Hi, *H. influenzae* antiserum.

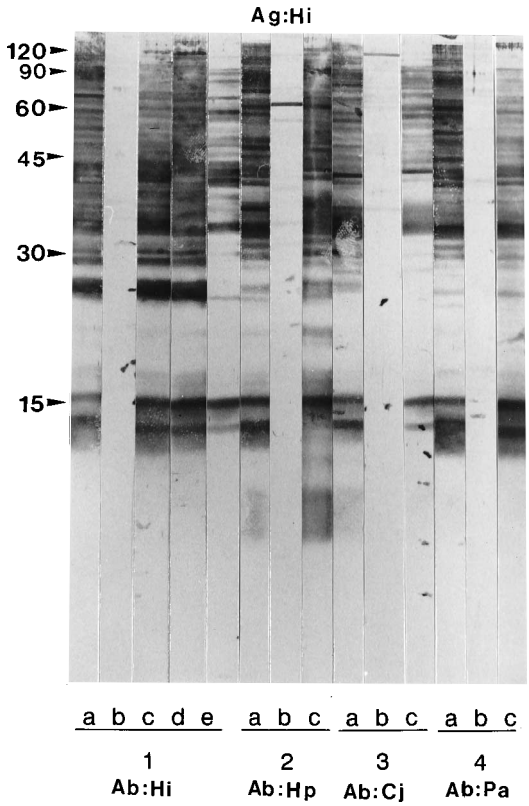

FIG. 4. Western blot analysis showing antibody responses to *H. influenzae* antigens. Lanes: 1a, unabsorbed *H. influenzae* antibody pool; 1b to 1e, *H. influenzae* antibody pool absorbed with *H. influenzae* (lane 1b), *P. aeruginosa* (lane 1c), *H. pylori* (lane 1d), and *C. jejuni* (lane 1e) antigens; 2a, unabsorbed *H. pylori* antibody pool; 2b and 2c, *H. pylori* antibody pool absorbed with *H. influenzae* (lane 2b) and *H. pylori* (lane 2c) antigens; 3a, unabsorbed *C. jejuni* antibody pool; 3b and 3c, *C. jejuni* antibody pool absorbed with *H. influenzae* (lane 3b) and *C. jejuni* (lane 3c) antigens; 4a, unabsorbed *P. aeruginosa* antibody pool; 4b and 4c, *P. aeruginosa* antibody pool absorbed with *H. influenzae* (lane 4b) and *P. aeruginosa* (lane 4c) antigens. Abbreviations: Ag:Hi, *H. influenzae* antigens; Ab:Hi, *H. influenzae* antiserum; Ab:Hp, *H. pylori* antiserum; Ab:Cj, *C. jejuni* antiserum; Ab:Pa, *P. aeruginosa* antiserum.

P. aeruginosa (lane 3c). The *H. influenzae* pool revealed several bands when tested against *C. jejuni* (lane 4a), and the 50-kDa band was only partially absorbed by *C. jejuni* antigens (lane 4b). A 56-kDa band and a 14-kDa band were absorbed by *H. influenzae* (lane 4c), indicating a cross-reaction.

Antibody response to *H. influenzae*. The immunoblot revealed a great number of bands when the *H. influenzae* pool was tested against *H. influenzae* antigens (Fig. 4, lane 1a), and complete absorption was found when the antibody pool was absorbed with *H. influenzae* antigens (lane 1b). No absorption was seen when the *H. influenzae* pool was absorbed with *P. aeruginosa* or *H. pylori* antigens (lanes 1c and d), whereas two bands of about 130 kDa and 29 kDa were absorbed with *C. jejuni* antigens (lane 1e), indicating a cross-reaction.

The *H. pylori* pool revealed a great number of bands when tested against *H. influenzae* (Fig. 4, lane 2a), and almost complete absorption was found when the antibody pool was absorbed with *H. influenzae* antigens (lane 2b). No absorption was seen when the *H. pylori* pool was absorbed with *H. pylori* antigens (lane 2c). Three bands of approximately 10, 12, and 20 kDa were stained stronger after absorption than before (lane 2c).

The *C. jejuni* pool also revealed a large number of bands when tested against *H. influenzae* antigens (Fig. 4, lane 3a), and almost all bands were removed after absorption with *H. influenzae* antigens (lane 3b). When the *C. jejuni* pool was absorbed with *C. jejuni* antigens, a 25-kDa band was removed (lane 3c), indicating a cross-reaction.

The *P. aeruginosa* pool revealed several bands when tested against *H. influenzae* (Fig. 4, lane 4a), whereas an almost complete absorption was seen by the *H. influenzae* antigens (lane 4b). No absorption was found with the *P. aeruginosa* antigens (lane 4c).

Identification of cross-reactions between *H. pylori* and *P. aeruginosa*. Monoclonal antibodies to the *P. aeruginosa*-specific part of the 60-kDa GroEL protein revealed a weak 60-kDa band with *P. aeruginosa* antigens and no band with *H. pylori* antigens (Fig. 5, lanes a and h). Monoclonal antibodies to a conserved part of *P. aeruginosa* 60-kDa GroEL revealed a strong 60-kDa band with *P. aeruginosa* antigens and also a band with *H. pylori* antigens (lanes b and g). Polyclonal rabbit antibodies to *P. aeruginosa* 60-kDa GroEL revealed several bands at about 14, 27 to 30, 47 to 50, and 60 kDa with *P. aeruginosa* and *H. pylori* antigens (lanes c and f). The *H. pylori* pool absorbed with *P. aeruginosa* 60-kDa GroEL tested against *H. pylori* antigens revealed no absorption, whereas that tested against *P. aeruginosa* antigens (lane d) revealed absorption of

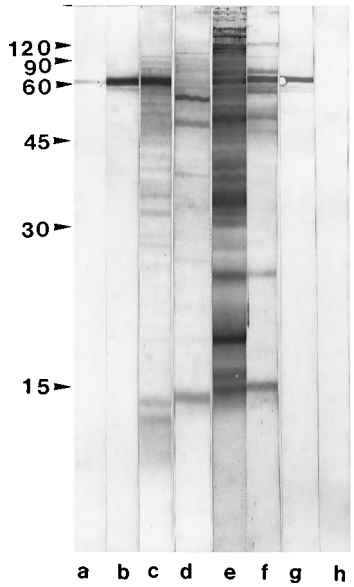

FIG. 5. Western blot analysis showing cross-reactions between *H. pylori* and *P. aeruginosa* antigens. Lanes: a, monoclonal antibodies (C2-F15) to the *P. aeruginosa*-specific part of 60-kDa GroEL; b, monoclonal antibodies (C2-F12) to a common, conserved part of *P. aeruginosa* 60-kDa GroEL; c, polyclonal rabbit antibodies to *P. aeruginosa* 60-kDa GroEL; d, *H. pylori* pool absorbed with *P. aeruginosa* 60-kDa GroEL when tested against *P. aeruginosa* antigens; e, *H. pylori* pool absorbed with *P. aeruginosa* 60-kDa GroEL tested against *H. pylori* antigens; f, polyclonal rabbit antibodies to *P. aeruginosa* 60-kDa GroEL; g, monoclonal antibodies (C2-F12) to a common, conserved part of *P. aeruginosa* 60-kDa GroEL; h, monoclonal antibodies (C2-F15) specific for *P. aeruginosa* 60-kDa GroEL.

the 60-kDa band but not that of the 14-, 27- to 30-, and 47- to 50-kDa bands (lane e).

DISCUSSION

In the present study, we found that sera from CF patients chronically infected with *P. aeruginosa* or *H. influenzae* or from non-CF patients recovering from acute *C. jejuni* infection cross-react with *H. pylori* antigens. The absorption studies show that these antibodies are directed against antigens which are common to several gram-negative bacteria, such as the 60-kDa heat shock protein. The cross-reactivity of the heat shock protein is in accordance with previous findings showing the wide occurrence of cross-reactive antigens in different bacteria (19). The number of common cross-reactive antigens and the degree of cross-reactions (presence of specific and cross-reactive epitopes on the same molecule) have been found to be significantly correlated with the phylogenetic relatedness of the bacteria (19).

In the immunoblot using *H. pylori*, *P. aeruginosa*, and *C. jejuni* antigens, the 14-kDa antigen was absorbed by all of the heterologous serum pools, indicating strong cross-reactivity. In contrast, none of the 14-kDa bands in the *H. influenzae* immunoblot was absorbed. This pattern could be explained by distant relatedness between *H. influenzae* (matching coefficient, 0.10) (19) and the other gram-negative bacteria in the present study. Generally, minor cross-reactive antigens were found at approximately 12, 30, 45, 70, 90, and 130 kDa.

When the *H. pylori* pool was absorbed with *H. pylori* and tested against *H. influenzae*, some of the antibodies were expressed more strongly after absorption than before. Although we detected only IgG antibodies, IgM and IgA antibodies (31) which cross-react with the absorbed antigens may compete by binding the antibody epitopes and thereby causing this phenomenon. Absorption of the *H. pylori* pool with *H. influenzae* antigens removed more bands than absorption with *H. pylori* antigens. This is not surprising considering that *H. influenzae* infections are very common and induce antibodies in virtually all persons (38, 39). A well-characterized antigen is the 60-kDa common protein antigen of *P. aeruginosa*, which is identical to GroEL (19, 24, 43). The attempt to identify cross-reacting antigens between *H. pylori* and *P. aeruginosa* by using monoclonal antibodies revealed that both *H. pylori* and *P. aeruginosa* contained a 60-kDa GroEL common protein antigen. Furthermore, the polyclonal rabbit antibodies to *P. aeruginosa* 60-kDa GroEL common protein antigen revealed several bands against *H. pylori* and *P. aeruginosa* at about 14, 27 to 30, 47 to 50, and 60 kDa, indicating corresponding cross-reactions between *H. pylori* and *P. aeruginosa* and maybe also the presence of cross-reactive antibodies from *Bordetella bronchisepticum*, which frequently infects rabbits (19). According to these results, the GroEL protein of *P. aeruginosa* and *H. pylori* is responsible for part of the cross-reacting antibodies which may give rise to false-positive antibody titers to *H. pylori* in CF patients (36).

Furthermore, it has been indicated that antibodies against LPS from several bacteria cross-react with *H. pylori* (2, 30, 34). A major cross-reaction due to the flagellar or common protein antigen of 52 or 56 kDa has been described previously between *H. pylori* and *C. jejuni* (30), which is in accordance with the findings in the present study. Antibodies in the *H. pylori* pool to the common protein antigen were absorbed by *P. aeruginosa* antigens. In contrast, complete absorption of all *P. aeruginosa* antibodies to the common GroEL antigen of *H. pylori* and *C. jejuni* could not be observed probably because of the large amount of antibodies in the *P. aeruginosa* pool to the *P. aeruginosa*-specific part of this antigen (19).

Patients with CF may suffer from gastrointestinal problems which are often associated with exocrine pancreatic dysfunction (22), but few studies have, until now, focused on the possibility of *H. pylori* being involved in the pathogenesis. Przyklenk et al. (36) presented evidence that gastritis due to *H. pylori* was not more common in CF patients than in non-CF controls. However, significantly elevated IgG titers were detected in two age groups, i.e., in children less than 12 months of age and in adults between 20 and 24 years of age. The elevated titers might be due to infection with *H. pylori*; it is, however, more likely that the increased titers in the small children (31) are caused by interfering cross-reacting 14- and 70-kDa protein antigens of *H. pylori* and *H. influenzae* as indicated in the present study (Fig. 1, lane 4c). In the adult CF patients (31), the elevated antibody response may be caused by cross-reacting *H. pylori* and *P. aeruginosa* protein antigens (14, 46 to 50, 56, and 61 kDa) (Fig. 1, lane 3c). It is, however, notable in the study by Przyklenk et al. (36) that 50% of all CF patients between 30 and 34 years of age had positive *H. pylori* titers compared with 30% of non-CF patients. This observation is in accordance with the occurrence of an increased number of *P. aeruginosa* precipitins in older CF patients (10) who are chronically infected and thereby an increased number of cross-reacting antibodies causing false-positive results. Such cross-reactive antigens have been shown previously to be responsible for high antibody titers to *Legionella pneumophila* in CF patients (6, 8).

In view of the present findings, a serological diagnosis of *H. pylori* infection in CF patients cannot be recommended unless purified non-cross-reacting antigens are employed. Previously, a 120-kDa (9) protein with mucosal IgA recognition was reported to possess pathogenic features associated with active

gastritis and peptic ulceration due to *H. pylori*. In another study, a combination of 19- to 36-kDa proteins was found to be specific for *H. pylori* with a high level of discrimination between *H. pylori*-positive and -negative patients (2). If *H. pylori* infection should be diagnosed or excluded in CF patients, the urease breath test or culture of the bacteria from gastric biopsies may be recommended.

In conclusion, the present study shows that *H. pylori* cross-reacts with several antigens from *P. aeruginosa*, *H. influenzae*, and *C. jejuni*, and increased antibody levels to *H. pylori* in patients with these infections cannot be regarded as indicating present or past *H. pylori* infection unless their specificity has been proven by absorption studies as exemplified in the present study.

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