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Antigenic Characterization of *Helicobacter pylori* Strains from Different Parts of the World

JOHANNA HÖÖK-NIKANNE,[1] GUILLERMO I. PEREZ-PEREZ,[1] AND MARTIN J. BLASER[1,2]*

Division of Infectious Diseases, Vanderbilt University School of Medicine,[1] and Veterans Affairs Medical Center,[2] Nashville, Tennessee

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Although *Helicobacter pylori* is considered to be relatively homogeneous at the phenotypic level, our aim was to describe its antigenic heterogeneity and to examine differences in host response. Whole-cell lysates of *H. pylori* strains originally isolated from persons from Africa, the People's Republic of China, Japan, Peru, Thailand, or the United States or from monkeys were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoblots were performed by using sera from *H. pylori*-infected persons from different areas of the world and rabbit immune sera against *H. pylori* antigens. Specific *H. pylori* antibody responses in persons from the United States and the People's Republic of China were analyzed by enzyme-linked immunosorbent assay with antigens prepared from U.S. or Chinese strains. Despite diverse origins, the strains showed conserved major bands of 84, 60, 56, 31, and 25 kDa. Although there were clear differences in minor bands, there was no obvious geographic pattern. The anti-CagA serum recognized 120- to 140-kDa bands in *cagA*$^+$ strains from around the world. Although antigenic preparations from individual U.S. or Chinese strains were not optimally sensitive for serologic detection of infection in the heterologous country, use of pools of strains largely overcame this phenomenon. We conclude that conserved *H. pylori* antigens exist and are recognized by sera from persons from many parts of the world. The heterogeneity of *H. pylori* antigens and the serological responses of infected hosts is not fully explained by geographic differences. Use of pools may allow development of antigens for serologic testing in any country.

Helicobacter pylori-induced gastritis is common worldwide (8), and previous studies in several parts of the world suggest that all *H. pylori* isolates have similar sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profiles (15–17, 20). In addition, growth characteristics, SDS-PAGE profiles of acid glycine extracts, and DNA base composition and enzymatic profiles have been found to be quite similar for a variety of *H. pylori* isolates (14, 16). However, within *H. pylori* strains there are clearly phenotypic differences, including production of the CagA protein (7, 27) and expression of a vacuolating cytotoxin (10). Phenotypic differences also have been demonstrated by coagglutination testing (11), and a study of serologic responses to *H. pylori* infection in Thailand (6) suggested that antigenic differences among *H. pylori* strains may have a geographic component. Information concerning *H. pylori* antigenic differences is important for selection of preparations used for serological tests and for possible vaccine development. For these reasons, more information about antigenic differences among *H. pylori* isolates is needed.

The goals of the present study were to assess the variability among *H. pylori* strains from different parts of the world in SDS-PAGE protein profiles, to identify antigens common to all strains, and to compare the serologic responses of persons from different populations to *H. pylori* antigens.

MATERIALS AND METHODS

Bacterial strains. We studied 21 *H. pylori* strains isolated from patients from the People's Republic of China, Japan, Peru, Morocco, Thailand, the United States, and Zaire and 3 strains from rhesus monkeys (Table 1). The strains were grown on plates with Trypticase soy agar and 5% sheep blood, and bacterial cells were collected in 0.9% saline, washed, and then stored at −20°C at a concentration of approximately 5 mg/ml. The strains were identified as *H. pylori* on the basis of colony morphology, Gram stain, and urease activity (16). The presence

TABLE 1. *H. pylori* strains used in this study

Origin	Strain	Clinical diagnosis[b]	Genotype		SDS-PAGE lane	Western blot lane
			cagA	*vacA*		
China[a]	95-10	Gastritis	+	s1m1	1	12
China	95-12	Gastritis	+	s1m2	2	11
China	95-13	Gastritis	+	s1m1	3	10
Japan	96-9	GU	+	s1am3	4	9
Japan	96-10	DU	+	s1m1	5	8
Thailand	88-28	ND	+	s1am2	6	7
Thailand	88-32	GU	+	s1m1	7	6
Thailand	88-39	ND	+	s1am2	8	5
U.S.[c]	95-46	Gastritis	−	s2m2	9	4
U.S.	95-60	Gastritis	+	s1bm2	10	3
U.S.	95-62	DU	+	s1am2	11	2
U.S.	84-183	ND	+	s1bm1	12	1
Monkey	96-11	Gastritis	+	ND	13	24
Monkey	96-12	Gastritis	−	ND	14	23
Monkey	96-13	Gastritis	+	ND	15	22
Africa	96-14	Gastritis	−	s2m2	16	21
Africa	96-15	GU	−	s2m2	17	20
Africa	96-16	DU	+	s1m2	18	19
Africa	96-17	DU	+	s2m2	19	18
Peru	90-12	Gastritis	+	s1m2	20	17
Peru	90-19	Gastritis	+	s1m1	21	16
Peru	90-40	Gastritis	+	s1m1	22	15
U.S.	88-22	ND	−	s2m2	23	14
U.S.	88-23	ND	+	s1am1	24	13

[a] China, People's Republic of China.
[b] GU, gastric ulcer; DU, duodenal ulcer; ND, not determined.
[c] U.S., United States.

* Corresponding author. Mailing address: Division of Infectious Diseases, Medical Center North A3310, Vanderbilt University School of Medicine, Nashville, TN 37232-2605. Phone: (615) 322-2035. Fax: (615) 343-6160. E-mail: Martin.Blaser@Mcmail.Vanderbilt.edu.

TABLE 2. Sera from *H. pylori*-infected persons used in this study by country of origin and ELISA values representing recognition of *H. pylori* whole-cell and CagA antigens

Country[a]	Serum designation	ELISA optical density	
		H. pylori whole-cell antigen[b]	CagA (status)[c]
U.S.	93-713	3.430	0.994 (+)
U.S.	88-557[d]	2.478	0.685 (+)
Mexico	87-743	4.111	0.142 (−)
Mexico	96-1979	2.587	0.844 (+)
China	87-142	1.128	0.348 (+)
China	94-403	1.152	0.626 (+)
Ethiopia	89-1	1.699	0.577 (+)
Ethiopia	89-8	1.179	0.362 (+)
New Zealand	88-2332	1.702	0.585 (+)
New Zealand	88-2343	1.716	0.566 (+)
Japan	88-1041	1.000	0.670 (+)
Japan	88-1034	3.210	0.783 (+)
Thailand	88-228	0.921	0.845 (+)
Thailand	88-217	2.135	1.009 (+)

[a] U.S., United States; China, People's Republic of China.
[b] Whole-cell antigen derived from five U.S. strains; cutoff for positivity, ≥1.00.
[c] CagA antigen derived from a U.S. strain; cutoff for positivity, ≥0.300.
[d] Obtained from a Native American of the Pima tribe.

of *cagA* and the *vacA* subtype of the strains had been determined as described previously (2, 28).

SDS-PAGE and immunoblotting. SDS-PAGE of whole-cell lysates using 10% acrylamide gels and silver staining was performed as previously described (4). Protein concentration (determined by bicinchoninic acid assay; Pierce, Rockford, Ill.) was adjusted to 2 mg/ml, 10 μl of each preparation was loaded per lane, and immunoblotting was performed as previously described (22). In brief, proteins from gels of whole-cell lysates of *H. pylori* were transferred to nitrocellulose paper by electroblotting at 1 A for 30 min. Immunoblotting was performed with rabbit or human sera diluted 1:100. Goat anti-rabbit or goat anti-human antibody-alkaline phosphatase conjugates (Biosource International, Camarillo, Calif.) diluted 1:2,000 were used. Immunoreacting bands were detected as previously described (19). In past studies, serum from uninfected animals or humans (20) showed essentially no recognition of the *H. pylori* antigens.

Sera for immunoblotting. Rabbit serum against a whole-cell preparation of *H. pylori* 84-181 had been prepared as outlined previously (20). The preparation of rabbit antiserum against a CagA recombinant protein has been previously described (9). Rabbit antiserum raised against the urease-heat shock protein B (HspB) complex of *H. pylori* 84-183 was prepared as reported earlier (12).

Human serum samples from *H. pylori*-infected persons from the United States (one Caucasian of European descent and one Native American), Asia (two each from the People's Republic of China, Japan, and Thailand), Latin America (two from Mexico), Africa (two from Ethiopia), and Oceania (two from New Zealand) were used. All of the serum samples recognized *H. pylori* antigens according to the criterion previously validated (optical density, >1.0) in the enzyme-linked immunosorbent assay (ELISA) developed for serodiagnosis of infection (21). The immune responses of these sera to CagA antigen are shown in Table 2, and a value of ≥0.300 is considered positive as previously validated (5).

Sera for ELISAs to detect antibodies to antigens from U.S. and Chinese *H. pylori* strains from patients. Serum samples obtained from two countries (the United States and the People's Republic of China) were compared for their immunoglobulin G (IgG) responses to antigen preparations from strains from the United States or the People's Republic of China. The U.S. serum samples were from 65 patients who underwent gastroscopy. *H. pylori* status was established by culture, histology, and PCR as described previously (18). The Chinese serum samples were from 40 patients from Shandong Province who had been determined to be infected or not infected with *H. pylori* based on urea breath tests (32).

Serological assays. Serum samples were tested for the presence of *H. pylori*-specific IgG by using a previously described ELISA (21). Four different antigen preparations were used at a concentration of 1 μg of protein/well as had been validated previously (20, 21). The U.S. single antigen was a sonicated whole-cell preparation of *H. pylori* 84-183 (isolated from a patient in Texas). The Chinese single antigen was derived from a sonicated whole-cell preparation of *H. pylori* 95-10 (a strain isolated from a patient in Shandong Province). The U.S. pooled antigen was derived from an equal mixture of sonicates from five U.S. strains (84-183, 86-86, 86-63, 84-181, and 84-182). These strains have been used to provide antigens for assays since 1986. The original freezer stocks have been subcultured every few years to provide new pools of antigens. By using reference sera, we have found no substantial change in their recognition of these antigens. The Chinese pooled antigen was derived from five Chinese strains (95-10, 95-11, 95-12, 95-13, and 95-14). All serum samples were diluted 1:800 and examined in duplicate wells on two different days. This single high dilution was selected to dilute out nonspecific antibodies; its accuracy for screening has been previously described (18, 21). Appropriate blanks and positive and negative controls were included in each assay as previously described (23, 24).

RESULTS

SDS-PAGE profiles. Despite the geographic diversity of the *H. pylori* strains studied (Table 1), their SDS-PAGE protein profiles showed many similarities, including conserved major bands at 84, 60, 56, 31, and 25 kDa (Fig. 1). Some of these bands represent major protein components of the bacteria, such as urease and the cpn60 heat shock protein (12, 13). Several regions in which differences also were detected are indicated by arrows pointing to bands at approximately 100, 97, 61, 35, and 18 kDa.

Immunoblotting. By immunoblotting, the rabbit anti-whole-cell serum recognized many of the same bands in most of the strains. However, diversity among the strains was clearly detected, particularly in the regions around 85 and 30 kDa (Fig. 2). The rabbit anti-CagA serum recognized high-molecular-mass bands (120 to 140 kDa) in each of the 19 *cagA*$^+$ strains (data not shown). As expected (12, 25), the antiserum to the heat shock complex recognized the conserved bands of 61, 56, 31, and 25 kDa in each of the strains studied (data not shown).

The 13 human serum samples studied showed substantial heterogeneity in their recognition of antigens from the panel of

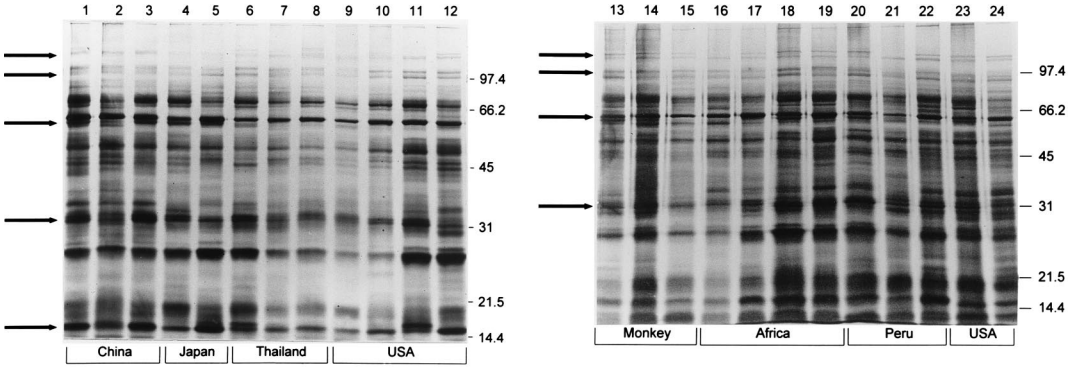

FIG. 1. SDS-PAGE profiles of 24 whole-cell preparations of *H. pylori* from patients and monkeys from different areas of the world. The lane for each preparation is indicated in Table 1, and the origins of the strains are indicated by the brackets below the lanes. The numbers on the right are molecular weights in thousands. The arrows on the left indicate areas of difference.

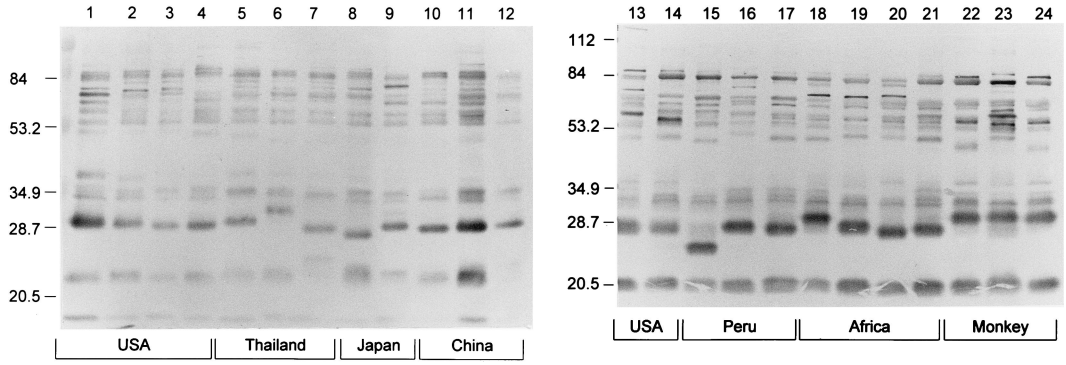

FIG. 2. Immunoblot results of *H. pylori* whole-cell preparations with rabbit antiserum to *H. pylori* 84-181. Molecular weight markers are indicated at the left, and the origins of the strains are shown in Table 1, as well as by the brackets below the lanes.

H. pylori strains from different parts of the world. Representative immunoblots are shown in Fig. 3. In general, each of the sera recognized a wide variety of antigens in each of the strains studied. Serum from a person of European descent in the United States (Fig. 3A) and that from a person in Latin America (data not shown) recognized a wide variety of bands in the strains, with clear recognition of the 61-, 56-, and 31-kDa antigens that represent the urease and heat shock proteins. This pattern is consistent in all of the strains tested, although recognition of several higher-molecular-mass bands was more variable. In contrast, sera from Japanese (data not shown) and Chinese (Fig. 3B) patients had a less homogeneous pattern of recognition. For nearly 50% of the strains, there was intense recognition of the region between 25 and 80 kDa, with minimal recognition of individual bands, and there was lack of recognition of the urease and heat shock antigens. The strains that were recognized best by sera from these Asian patients were not clustered geographically, and even for strains from the same region, the sera showed different recognition patterns. The serum from an Ethiopian person (Fig. 3E) recognized the urease bands in most, but not all, of the strains, and for some strains it had more intensive recognition of a region from 30 to 50 kDa, but this phenomenon was less pronounced than with the sera from the Asian patients. The sera from both of the Maori persons (Fig. 3C shows one) and the Pima Indian (Fig. 3D) had poor recognition of bands between 20 and 50 kDa, but recognition of high-molecular-mass bands in all of the strains was similar. Thus, as exemplified by the five serum samples shown in Fig. 3, the human IgG serological response to *H. pylori* antigens is highly heterogeneous.

Serology. The use of a single *H. pylori* strain as an antigen for ELISAs to detect human serum antibodies would be expected to have low diagnostic value, especially when the origins of the strain and the sera to be tested are not similar. However, use in serum IgG ELISAs of whole-cell lysates from single strains from the United States and the People's Republic of China gave remarkably good differentiation between infected and uninfected persons, although detection of antibodies in sera from infected persons in the homologous region was greater (Table 3). When pools of strains from either the United States or the People's Republic of China were used as the source of antigen, they were highly sensitive and specific for detecting infection based on sera from the same locality, and their performance for classification of the heterologous sera was similar (Table 3).

DISCUSSION

H. pylori strains are highly diverse at the genetic level (3), and there is evidence of phenotypic diversity as well (1, 29, 30). Since *H. pylori* infection is cosmopolitan, it is important to understand the antigenic variation of *H. pylori* strains in different parts of the world. This point is particularly significant for the development and use of serologic tests to detect the presence of infection, as well as for development of vaccines. For these reasons, we sought to survey both strains and human sera from different parts of the world both to identify conserved antigens and to assess the degree and significance of antigenic variation. Since an estimated 3 billion persons worldwide are infected with *H. pylori* (26), our survey could not be comprehensive but we sought to include strains and sera from different representative geographic regions of the world. Inclusion of materials from New Zealand and the United States allowed comparison of indigenous (Pima Indian, Maori) and European-ancestry populations.

As anticipated by previous studies from particular localities (6, 15, 20), both the proteins and the antigens present in *H. pylori* strains have both conserved and diverse features. In particular, the proteins associated with the urease-HspB complex (migrating at 60, 56, 31, and 25 kDa) are well conserved antigens in all strains. The CagA protein also is a well-conserved antigen among strains from different localities, despite size heterogeneity (7, 27), but is not present in all strains. Nevertheless, our data indicate that CagA proteins from strains around the world can be detected by immune antiserum raised to the protein from a single strain and thus are closely related.

In contrast, individual *H. pylori* strains possess antigens that are not conserved, because of either presumed size variation or absence of the protein, as best indicated by Fig. 1 and as exemplified by previous studies of CagA expression and antigenicity (5, 9, 27, 31). This diversity was not sharply defined by geographic region, and even isolates from monkeys were as similar to human strains as human strains were to one another.

FIG. 3. Immunoblots of *H. pylori* preparations with sera from representative patients from different parts of the world. Molecular weight markers are indicated at the left, and the origins of the strains are shown in Table 1, as well as by the brackets below the lanes. Serum sources: A, United States (Caucasian, European descent); B, Asian (Chinese); C, New Zealand (Maori); D, Native American (Pima); E, African (Ethiopia).

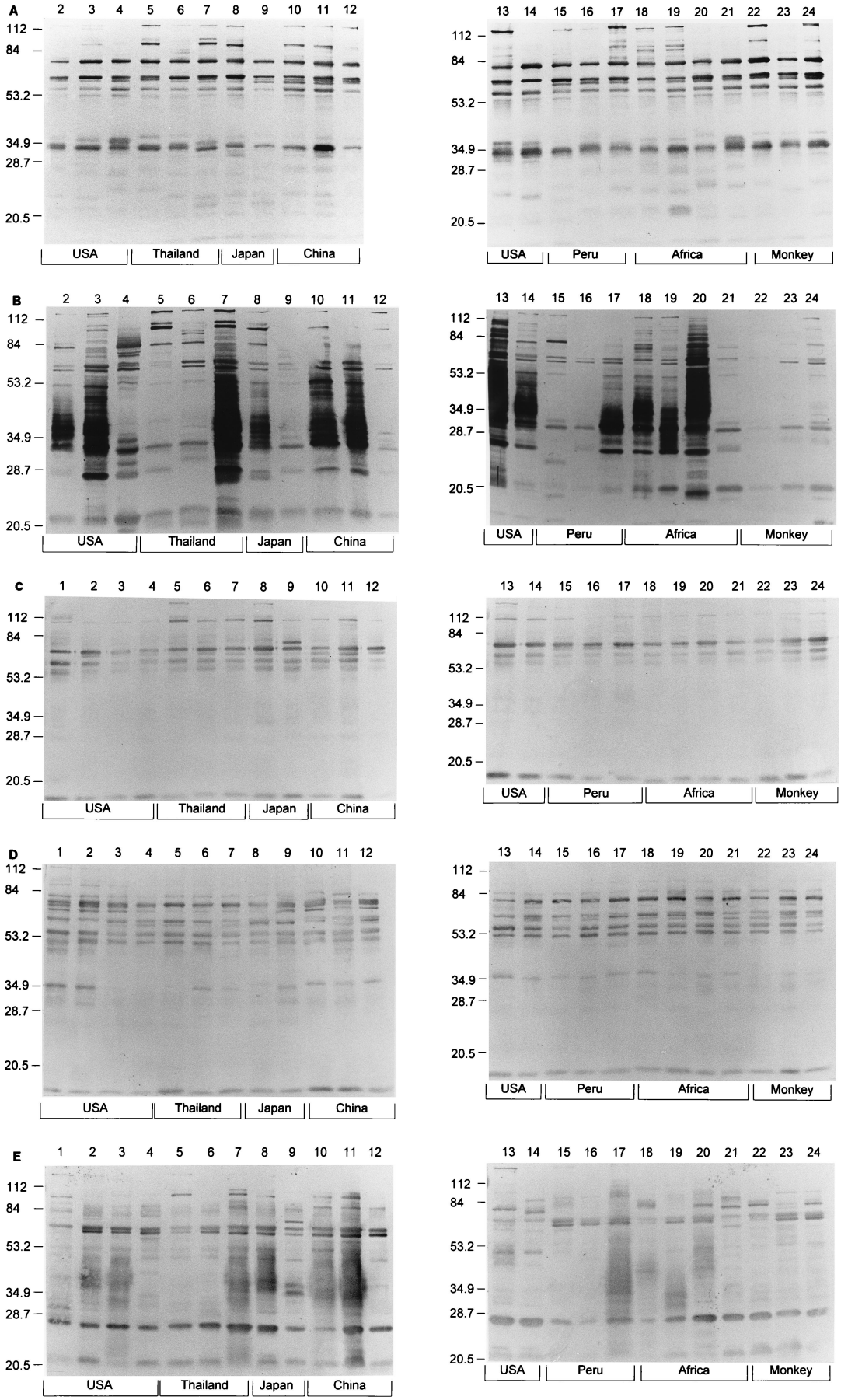
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TABLE 3. Serological responses of *H. pylori*-infected and uninfected U.S. and Chinese patients to local and foreign antigens[a]

Patient group[b]	*H. pylori* infection status	No. of serum samples tested	% Positive by source of *H. pylori* antigen				Mean optical densities by source of *H. pylori* antigen			
			Single strain[c]		Pool[d]		Single strain		Pool	
			U.S.	China	U.S.	China	U.S.	China	U.S.	China
U.S.	+	40	90	95	100.0	100.0	1.66 ± 0.08[e]	1.29 ± 0.03	3.19 ± 0.14	3.95 ± 0.19
U.S.	−	25	0	0	4.0	8.0	0.011 ± 0.02	0.15 ± 0.04	0.33 ± 0.06	0.40 ± 0.08
China	+	26	92.3	100.0	84.6	100.0	1.87 ± 0.17	2.86 ± 0.12	2.03 ± 0.18	2.89 ± 0.17
China	−	14	0	28.6	0	7.1	0.037 ± 0.06	0.65 ± 0.11	0.35 ± 0.07	0.45 ± 0.11

[a] In each assay, the cutoff for positivity was an optical density of >1.0.
[b] U.S., United States; China, People's Republic of China.
[c] The antigen used was derived from a single strain from the United States (84-183) or the People's Republic of China (95-10) and used at a concentration of 1 μg/well.
[d] The antigen used was derived from a pool of five strains from the United States (84-183, 86-86, 86-63, 84-181, and 84-182) or the People's Republic of China (95-10, 95-11, 95-12, 95-13, and 95-14) and used at a concentration of 1 μg/well.
[e] Each comparison using a particular antigen significantly ($P < 0.001$) discriminated between infected and uninfected persons.

These data suggest that within particular geographic regions, considerable antigenic diversity of *H. pylori* strains exists. We did not find evidence that major Asian, African, New World, or European clones are circulating; definitive examination of that hypothesis must come from genetic studies.

The human serologic response to *H. pylori* also showed the presence of antibodies to both conserved and diverse antigens. The main observation is that the responses were quite heterogeneous, and the sample examined indicated few clear-cut patterns. The data in Fig. 3 suggest that regional differences in the exact antigens recognized and the pattern of recognition may exist, but this phenomenon cannot be firmly established by the present studies; further analyses that extend these observations are warranted.

Given the heterogeneity of both *H. pylori* strains and the serologic responses to these organisms around the world, an important question is whether this diversity is clinically significant in relation to serologic detection of infection. The studies using antigens prepared from a single strain from the United States or the People's Republic of China and examining responses from persons in the United States (predominantly of European ancestry) and the People's Republic of China demonstrate that despite the heterogeneity of the immunoblots, the ELISAs indicate a very high degree of antigenic similarity among the strains. Heterogeneity was observed, but the fact that the use of pools restored sensitivity to the assays without substantial loss of specificity suggests that it is possible to develop common pools for use in serologic testing around the world. These data further suggest that there exists a conserved human serological IgG response to *H. pylori* antigens, despite antigenic variability, which may reflect stereotypic biological interactions between host and parasite. The stability of serological responses in individual persons to both group and specific *H. pylori* antigens (5) over many years (in the absence of treatment) is another indication of the homeostatic nature of colonization by *H. pylori.*

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