INFECTION AND IMMUNITY, Jan. 1997, p. 49–54 Vol. 65, No. 1
0019-9567/97/$04.00+0

Successful Cultivation of a Potentially Pathogenic Coccoid Organism with Trophism for Gastric Mucin

SUNG G. LEE,[1]* CHANGSUNG KIM,[1] AND YOUNG CHUL HA[2]

Mogam Institute, Kyong-gi-do, Yong-in-Kun,[1] and Seoul National University,[2] Seoul, Korea

Received 24 May 1996/Returned for modification 13 August 1996/Accepted 9 October 1996

We have devised a procedure that permits the cultivation of a gram-positive coccoid species from biopsy material obtained from the antrum of the stomachs of patients with gastric disorders. Antibodies directed against surface proteins obtained from the coccoid isolates were detected in all patients with gastric disorders examined in this study, including both *Helicobacter pylori*-infected and *H. pylori*-uninfected patients. Several of these isolates, including a prototype designated strain SL100, have been characterized in some detail. Strain SL100 exhibits urease and exceptionally high catalase activities and assumes a variety of spherical morphologies as detected by electron microscopy. This isolate expresses an adhesin that binds to gastric mucin. The adhesin activity was detected only after the isolate was exposed to an acidic pH, suggesting that in the natural process of infection, the low pH of the stomach unmasks a cell surface component with adhesin activity. Strain SL100 grows best under a microaerophilic conditions (10% CO_2, 5% O_2, 85% N_2), but it also grows quite well under aerobic conditions. Thus, this organism would be expected to proliferate outside of the human host as well as in the gastric mucosa. Oral infection of newborn piglets resulted in colonization of the gastric antrum and growth retardation. Preliminary taxonomic classification indicates similarity to the *Staphylococcus* DNA homology groups containing *S. cohnii* and *S. xylosus*. One of us (C.K.) apparently became infected with this organism as indicated by gastric symptoms and the subsequent presence of strain-specific antisera not present in other workers in the laboratory.

A large proportion of the gastric disorders occurring in industrialized countries have been attributed to infections by *Helicobacter pylori* (11). However, some patients suffering from these symptoms are culture negative and antibody negative for *H. pylori*, and these disorders are often attributed to other causes, such as the habitual use of certain medications (11). In one study (9), an uncharacterized coccoid organism was proposed as the causative agent for gastric inflammation in some (8.5%) of the patients who underwent biopsy. In another study, Bode et al. (1) observed both the well-characterized spiral-shaped *H. pylori* and a coccoid form in a gastric biopsy specimen and proposed that *H. pylori* can assume a coccoid morphology. In these two studies, the coccoid forms could not be cultivated and for this reason no further characterization was possible. Several groups have observed that in old cultures or in an aerobic atmosphere, the motile, spiral-shaped *H. pylori* changes into a nonmotile coccoid form (2, 6, 15). It has been speculated that these coccoid forms appear under these unfavorable conditions as an infectious agent that can survive outside the human host. Experimental proof of a role of the coccoid forms in the transmission of infection has not been possible because the coccoid forms could not be cultivated in the laboratory. We have now isolated and cultivated a coccoid organism from biopsy material obtained from patients suffering from gastritis. Antibodies to the coccoid forms were detected in patients who were either culture positive or culture negative for *H. pylori*. Infection and colonization of porcine gastric antrum by *H. pylori* have been well established (3–5, 17). We report here that our prototype isolate, designated strain SL100, also infects the porcine gastric antrum and binds to porcine gastric mucin in vitro. We found that oral administration of the coccoid organism to neonatal piglets resulted in severe growth retardation, and coccoid organisms were detected and cultivated from the antral mucin of these piglets. These observations suggest that the coccoid organisms isolated from these patients with gastric symptoms may be human pathogens. The relationship of our coccoid isolates to *H. pylori*, if any, is unknown.

* Corresponding author. Present address: Department of Chemistry, City College of New York, Convent Ave. & 138th St., New York, NY 10031. Phone: (212) 650-6934. Fax: (212) 650-7974.

MATERIALS AND METHODS

Growth of bacteria. Blood agar medium was prepared containing an equal mixture of Columbia medium and Difco Tryptic Soy Broth supplemented with 7% sheep blood, and the medium also contained colistin (2,500 U/liter) and vancomycin (10 mg/liter). Mucosal tissue samples 3 to 4 mm in diameter were obtained by endoscopy from the antrum region of patients with gastritis who were admitted to St. Vincent Hospital, Suwon, Korea. The tissue samples were immediately partially embedded approximately 2 to 3 mm deep in the agar of a blood agar plate containing colistin and vancomycin and incubated in a 37°C chamber under microaerophilic conditions (10% CO_2, 5% O_2, 85% N_2). A ring of gray to white bacterial growth that appeared around the lodged tissue samples after 2 days of incubation was transferred to a fresh plate and spread to cover a surface area of 2 to 3 cm^2. This plate was incubated for 1 day at 37°C, and the heavy bacterial lawn formed was spread to cover the entire surface of the 82-mm-diameter agar plate. This plate was incubated overnight, after which a heavy lawn of bacterial growth covered the entire surface of the plate. *Staphylococcus aureus* and *Streptococcus faecalis* were grown on antibiotic-free blood agar plates at 37°C under microaerophilic conditions.

Binding of bacteria to porcine mucin. A lawn of coccoid forms was suspended in distilled water, and the cell density was adjusted to an optical density at 600 nm (OD_{600}) of 2. The cell suspension (500 μl) was mixed with 500 μl of buffer at pH 2.5 (10 mM HCl), 3.5 (20 mM formate buffer), 4.5 (20 mM acetate buffer), 6.0 (20 mM succinate buffer), or 7.5 (20 mM Tris-HCl) and incubated at room temperature for 20 min, and the cells were pelleted by centrifugation for 5 min at 10,000 rpm in an Eppendorf microcentrifuge. The cell pellets were suspended in 800 μl of 20 mM acetate buffer (pH 5.0), and 200 μl of porcine gastric mucin preparation linked to horseradish peroxidase was added to each tube. These mixtures were incubated at room temperature for 30 min, and the cells were pelleted by centrifugation. The pellets were washed three times in 1 ml of 20 mM acetate buffer (pH 5.0) and resuspended in 600 μl of 20 mM Tris-HCl buffer (pH 7.8). The cell-bound mucin was determined by measuring peroxidase activity, using 0.05% 4-chloro-1-naphthol and 0.03% H_2O_2 for color development. To link horseradish peroxidase to mucin, 1 g of porcine gastric mucin (type II [Sigma]) was dissolved in 20 ml of 0.02% trypsin in 20 mM Tris-HCl buffer, and

the mucin was digested for 3 h at 37°C. The digested mucin was centrifuged for 30 min at 15,000 rpm with a Sorvall centrifuge and SS34 rotor, and the supernatant was filtered over a Sephadex G-200 column equilibrated with 10 mM phosphate buffer (pH 7.2). The material eluting at the void volume was concentrated to 10 ml and covalently linked to horseradish peroxidase with *N*-succinimidyl-3-(2-pyridylthio)propionate as a linker, as specified by the supplier (Pierce, Inc.).

Isotopic labeling of bacterial surface proteins. Coccoid cells were suspended in distilled water, the cell density was adjusted to an OD_{600} of 2, and cells in 1-ml aliquots were pelleted in two Eppendorf microcentrifuge tubes. The cell pellet in one tube was suspended in 1 ml of distilled water, and the pellet in the second tube was suspended in 1 ml of 10 mM HCl. After standing for 20 min, the cells were pelleted by centrifugation. The pellets were resuspended in 20 mM phosphate buffer (pH 7.2), and the surface-exposed proteins were labeled with ^{125}I as described previously (12). ^{125}I-labeled cells were collected and free ^{125}I was removed by repeated centrifugation and suspension of cell pellets. The final cell pellets were suspended in 10 mM Tris-HCl (pH 7.0) containing 0.5% sodium dodecyl sulfate (SDS) and 1 mM dithiothreitol, the suspension was boiled for 10 min, and the cells were removed by centrifugation. The extracted proteins in the solutions were separated by SDS-polyacrylamide gel electrophoresis (PAGE) with a 12% acrylamide gel, and an autoradiogram was made from the dried gel.

Binding of bacteria to porcine gastric antrum sections. The antrum region was cut from the stomach of a freshly sacrificed 7-day-old piglet, and the tissue was thoroughly washed with tap water. Tissue sections of about 7 by 7 mm were cut from the antrum and dipped in 0.1% hypochlorite solution for 5 s. The sections were then washed with phosphate-buffered saline (PBS) by five serial transfers of the sections into fresh PBS solution. Mucosa and submucosa were scraped from the tissue section with a scalpel, and the scraped tissue material was introduced into microcentrifuge tubes containing 1 ml of suspension of the gastric coccus or *S. aureus* in 20 mM formate buffer (pH 3.5). The mixtures of antral tissue material and bacteria were gently shaken for 1 h at room temperature. They were then pipetted to disperse tissue aggregates and were overlaid on 10% sucrose in 20 mM acetate buffer (pH 5.0) in a 15-ml conical tube. Tissue material was allowed to settle by gravity. The tissue materials were stained with neutral red, and the stained tissue materials were observed under a microscope.

Octylglucose extraction of bacterial cell surface proteins. Two days after reaching confluence, the cells were scraped off the plates and suspended in distilled water, and the cell density was adjusted to an OD_{600} of about 5 with distilled water. Aliquots of the cell suspensions (1 ml) were transferred to microcentrifuge tubes, and the cells were pelleted by centrifugation. The cell pellets in each tube were suspended in 1 ml of 2% octylglucose in 20 mM Tris-HCl buffer (pH 8.4), and the suspensions were incubated for 2 h at 42°C. At the end of the incubation, cells were pelleted by centrifugation and supernatants were collected in microcentrifuge tubes. To each supernatant, trichloroacetic acid was added to a final concentration of 5%, and the resulting protein precipitates were pelleted. The protein pellets were washed with ethanol and then dissolved in SDS-PAGE sample buffer, and the proteins were separated by SDS-PAGE with a 10% acrylamide gel. Protein bands were stained with Coomassie blue for visualization.

Demographics of patients examined by Western blot analysis and biopsy techniques. We obtained the sera, gastric antrum biopsy samples, and histological recordings of gastric antra from 18 patients with gastric symptoms who were admitted to Dankook University Medical School, Chonan, Korea. The sera were examined for the presence of antibody to *H. pylori*, and the biopsy samples were examined for the presence of *H. pylori*. Twelve patients were antibody positive and culture positive for *H. pylori*; the other six were antibody negative and culture negative for *H. pylori*. For Western blot analysis, we chose the sera of five patients in the first group and four patients in the second group. The histological status, age, and sex of the serum donors for the experiment presented in Fig. 6 are as follows: lane 1, chronic superficial ulcer, age 35, female; lane 2, multiple superficial ulcers, age 64, female; lane 3, erosive gastritis, age 64, female; lane 4, superficial gastritis, age 36, female; lane 5, gastritis, age 33, male; lane 6, superficial gastritis, age 32, male; lane 7, chronic benign gastric ulcer, age 56, male; lane 8, duodenal ulcer, age 36, male; lane 9, duodenal ulcer, age 39, male.

To obtain biopsy samples, gastroenterologists scanned the antral regions of the patients with an endoscope to search for inflamed regions and took samples from the lesions. The sampling scoop at the tip of the endoscope was closed during the entry into the stomach and throughout scanning, and it opened briefly only during sample taking. A fresh, sterilized endoscope was used for each patient. The entire process was displayed on a monitor during the procedure. The histological diagnoses were based on endoscopic observations, and in some cases the results of examination of tissue samples were examined in the pathology laboratory. No information about prior medications used by these patients is available. These patients were unrelated to each other, belonged to a relatively low income group, and were from an area which had been a farming community during the 1960s but had gradually turned into a manufacturing town during the 1980s.

Western blots of bacterial protein extracts. The cell surface proteins prepared and separated by SDS-PAGE as described above were transferred by electrophoresis to a nitrocellulose sheet. Strips with transferred protein bands were cut from the nitrocellulose sheet, and the strips were blocked with 2% skim milk in PBS. Each strip was then incubated overnight at room temperature with serum from an individual patient; the serum was diluted 100-fold with PBS. After several washes with PBS, the strips were incubated with a commercial horseradish peroxidase-linked goat anti-human immunoglobulin G (IgG) (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) which was diluted 1,000-fold with PBS. The strips were washed with several changes of PBS solution, and the protein bands recognized by serum IgG of the patients were detected by incubating the strips in a PBS solution containing 0.05% diaminobenzidine and 0.03% H_2O_2.

Serological testing and cultivation of organisms. The absence or presence of serum antibody to *H. pylori* (IgG^- and IgG^+, respectively) was measured as specified by the supplier of the diagnostic kit (Bio-Rad; no. 4404-2002). To determine the presence or absence of *H. pylori* in the gastric biopsy material, finely macerated biopsy material was cultured on blood agar plates under microaerophilic conditions (as described above), and the translucent colonies that appeared after 5 days were examined for the presence of spiral motile organisms with strong urease activity. For positive identification of *H. pylori*, aqueous extracts of the colonies were analyzed by SDS-PAGE for the presence of the 61- and 30-kDa urease subunits as described previously (13). Fatty acid analysis with the MIDI/Hewlett-Packard microbial identification system and metabolic characterization with the Biolog identification system were carried out by Analytical Services, Inc. (Williston, Vt.).

RESULTS

Isolation of the organism from biopsy material. We isolated a coccoid organism from the antrum region of the stomach of patients with gastric disorders by using endoscopy, and we cultured the organism on a standard blood agar medium which is commonly used to culture gastric organisms such as *H. pylori*. The culture was successful when the biopsy samples were partially embedded in blood agar plates. A ring of bacterial growth occurred around the embedded biopsy samples in 2 days. In contrast, there was no growth when the biopsy sample was ground into fine pieces and agitated vigorously before being spread to cover a culture plate. Similarly, growth was observed when the organism was spotted onto a plate at a high density (10 μl; OD_{600} = 10) but not at low density (10 μl; OD_{600} = 0.1). We suspect that the coccoid cells were present in clusters in the unbroken biopsy samples as judged by the clusters of coccoid cells seen in the antral mucosa of an infected piglet and in the culture on blood agar (Fig. 1). Taken together, these observations suggest that the organism grows best when cells are close to each other, as in cell clusters or in a heavy inoculum.

The organism exhibits strong urease and catalase activities, as does *H. pylori*. Presumably urease enables the gastric microorganism to survive in an acidic environment by producing ammonia (7, 16) while catalase enables the organism to overcome the assault of peroxides, which arise from the oxidative burst by host macrophages and neutrophils recruited to the submucosa underlying the infected mucosa (8, 14).

Scanning electron microscopy. Scanning electron microscopy revealed that the organism assumes a variety of morphologies. A spherical shape was most often observed, but in older cultures some of the spheres appeared to be dented (Fig. 1a), and in some cases the dents became so deep that the organism appeared doughnut-shaped (Fig. 1b). Occasionally, the coccoid forms were observed singly, but more frequently, they were seen in chains or clusters. When they were in a chain, it appeared as if a number of coccoid forms were encased in a stretchable outer coat, which held individual coccoid forms together.

Experimental infection of newborn piglets. A porcine system has been well established as a valid model for human infection with *H. pylori*. Therefore, we used the porcine system as a model for the human system for studying infection by our coccoid isolate. Two newborn piglets were given the coccoid-form organisms orally. At 4 days postinfection, one of the infected piglets was sacrificed, and tissue sections were made from the stomach. The tissue sections were washed and then

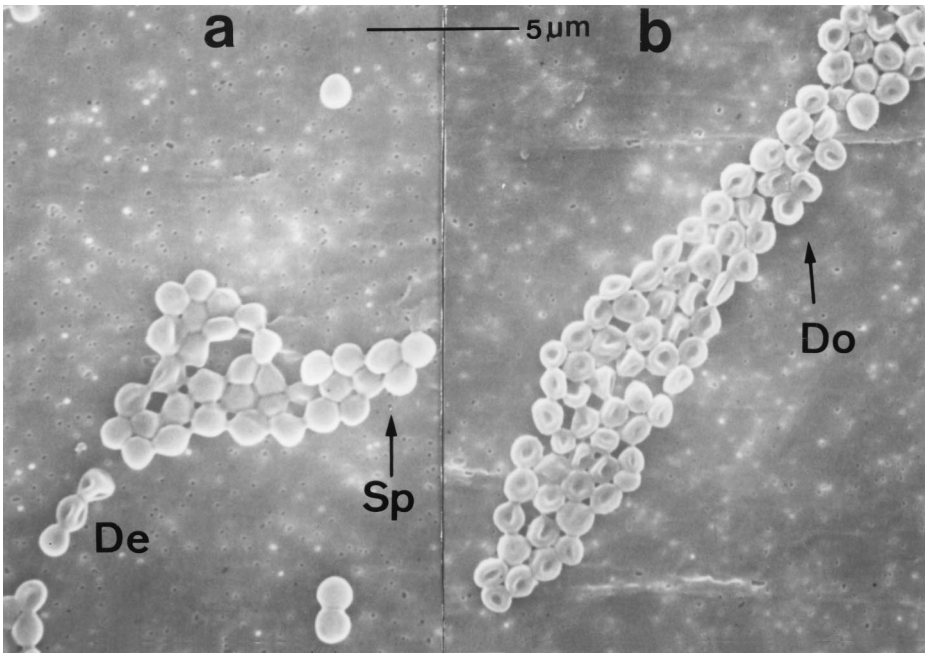

FIG. 1. Various morphologies of strain SL100 observed by scanning electron microscopy. (a) Dented (De) and spherical (Sp) cells; (b) dented and doughnut-shaped (Do) cells.

fixed with glutaraldehyde. When the fixed tissue sections were examined with a microscope, the mucin layer of the antrum sections was found to be heavily infected with the coccoid forms, but no other organism was detected in this layer. The coccoid forms were present in patches and in large clusters, rather than uniformly dispersed, on the mucin layer of the antrum. The growth of the two infected piglets was severely retarded compared to that of uninfected littermates. At 4 days after birth, the two infected piglets weighed 0.61 and 0.64 kg, while the average weight of the six uninfected littermates was 0.96 ± 0.07 kg.

Acid-dependent binding to porcine mucin. After observing a heavy infection of the porcine antrum by the coccoid organisms, we considered the possibility that colonization depends on the low pH prevailing in the stomach. Therefore, we examined the effect of acid exposure of the coccoid forms upon their binding to porcine mucin. To detect cell-bound mucin, horseradish peroxidase was covalently linked to the mucin preparation. As shown in Fig. 2, the coccoid-form bacteria bound to porcine gastric mucin only if they were preexposed to a pH of 3.5 or lower, which approaches the prevailing pH of the human and porcine stomach (3, 18). The binding of acid-treated coccoid forms to mucin was also dependent on the ambient pH. Thus, the acid-treated coccoid forms bound avidly to the mucin preparation at pH 5, but the binding of the same coccoid forms to the mucin preparation at pH 7.0 was greatly diminished. With the present experimental protocol, it was technically difficult to carry out the binding assay at a pH below 5 because the unbound horseradish peroxidase-linked mucin preparation tended to flocculate and coprecipitate with the coccoid forms at these pH values.

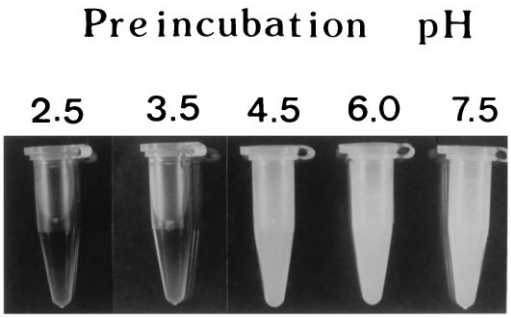

FIG. 2. Effect of acid exposure on strain SL100 upon binding to porcine gastric mucin preparations. The reaction is strongly positive (opaque appearance in the figure) in the tubes containing bacteria previously incubated at pH 2.5 and 3.5, weakly positive for bacteria previously incubated at pH 4.5, and negative for bacteria previously incubated at pH 6.0 or 7.5.

Detection of surface proteins after acid treatment. To examine whether acid treatment of the coccoid forms disrupts their surface organization, thereby unmasking an adhesin, the surface-exposed proteins of acid-treated and untreated coccoid forms were compared. For this purpose, the cell surface proteins of acid-treated and untreated coccoid forms were labeled with ^{125}I by the lactoperoxidase method (12). As may be seen in Fig. 3, acid treatment of the coccoid forms exposed at least four additional proteins with molecular masses of 17, 19, 25, and 34 kDa.

The gastric coccoid isolates have an exceptionally strong outer integument. Boiling strain SL100 with 0.5% SDS during extraction of ^{125}I-labeled cell surface proteins did not alter the integrity of the cells when examined under the microscope, and no lysis was detectable as evidenced by the absorbance at 260 nm of the medium. Cells treated with 10 mM HCl for 20 min grew equally well as did untreated cells when grown on blood agar medium.

Binding to porcine gastric mucosal scrapings. Various coccoid organisms, including a number of species of *Staphylococcus* and *Streptococcus*, are known to colonize the pharyngeal and esophageal mucin of warm-blooded animals. However, colonization of gastric antrum by these well-characterized

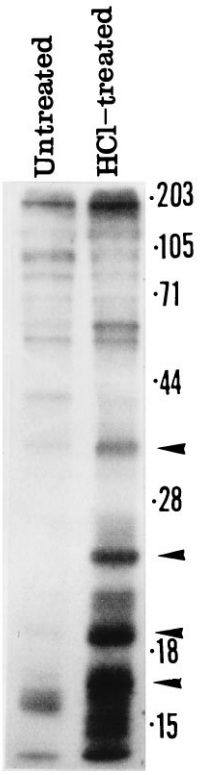

FIG. 3. SDS-PAGE analysis of radioiodinated surface proteins of untreated and HCl-treated gastric coccoid isolate. Arrowheads indicate the proteins that become exposed on the cell surface after acid treatment.

cocci has not been reported. To see if any of these cocci can bind to the antrum at an acidic pH, scrapings of mucosa prepared from the antrum of a 7-day-old piglet were incubated with our gastric coccoid forms or with *S. aureus* at pH 3.5. The results of this experiment (Fig. 4) seem to indicate that (i) our gastric coccoid forms bind mainly to the mucin but not to the epithelial cells of the piglet gastric antrum and (ii) *S. aureus* binds neither to mucin nor to epithelial cells of the antrum at pH 3.5.

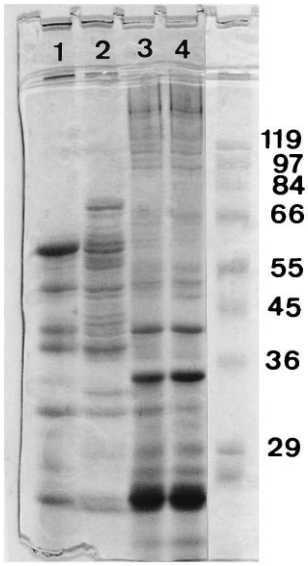

FIG. 5. Protein profiles of the octylglucose extracts of two gastric coccoid isolates compared to *S. aureus* and *Streptococcus faecalis*. Lanes: 1, *S. aureus*; 2, *Streptococcus faecalis*; 3 and 4, two gastric coccoid isolates.

Octylglucose extraction of surface proteins. Since microbial species exhibit characteristic profiles of cell surface proteins, we examined the cell surface protein profiles of the coccoid isolates. Figure 5 shows protein profiles of octylglucose extracts from two isolates of the coccoid forms (lanes 3 and 4) after SDS-PAGE and Coomassie blue staining. Lanes 1 and 2 show, as controls, protein profiles of *S. aureus* and *Streptococcus faecalis*, respectively. The profiles of octylglucose-extractable

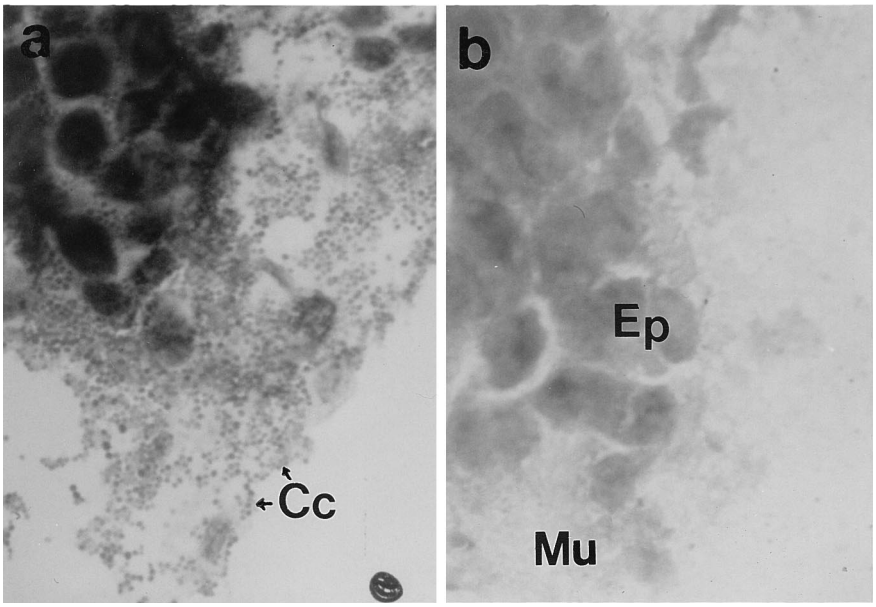

FIG. 4. Binding of strain SL100 (a) but not of *S. aureus* (b) to porcine gastric antrum. Porcine gastric mucosa was incubated with the gastric coccoid isolate strain SL100 or with *S. aureus*. Ep; epithelial cells, Cc; coccoid forms, Mu; mucin layer.

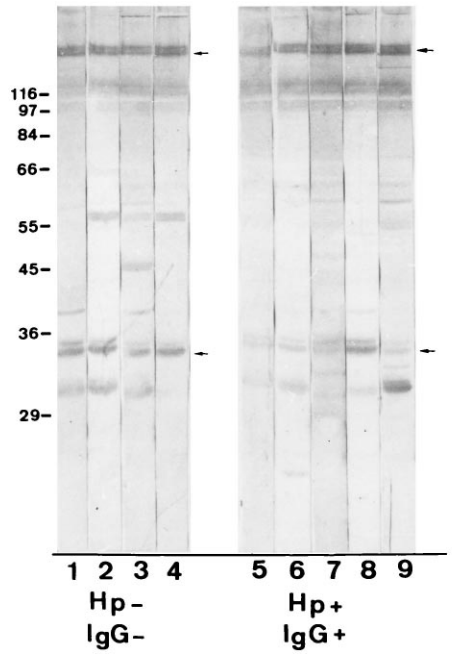

FIG. 6. Detection of antibodies in the serum of patients with gastric symptoms directed against proteins extracted from strain SL100. The proteins extracted by octylglucose were separated by SDS-PAGE on a 10% acrylamide gel and transferred to nitrocellulose for a Western (antibody) blot. Hp^- and Hp^+, culture negative and culture positive, respectively, for *H. pylori*; IgG^- and IgG^+, absence and presence of antibody to *H. pylori*, respectively. The patients are identified by the numbers at the bottom of each lane. The arrows indicate proteins recognized by the antibodies present in most or all patient serum samples.

proteins were qualitatively similar for these two independent coccoid isolates and are distinguishable from the patterns seen for *S. aureus* and *Streptococcus faecalis.*

Western blot analysis with serum from *H. pylori*-positive and -negative patients with gastric disorders. To determine if the coccoid organism is associated with gastric disorders, we tested antisera from patients with gastric ailments in a Western blot analysis with octylglucose-extracted proteins of a representative gastric coccoid isolate (strain SL100). From a group of 18 patients with gastric disorders, we selected the sera of 4 patients who were antibody and culture negative for *H. pylori* and the sera of 5 patients who were antibody and culture positive for *H. pylori*. Figure 6 shows the serum IgG status of these patients with respect to the protein components of the octylglucose extract made from the coccoid isolate shown in lane 4 of Fig. 5. Figure 6 clearly shows that all of these patients had serum IgG against the 34-kDa protein and the doublet of 200-kDa proteins and that the overall profiles of the serum IgG from the *H. pylori*-infected and those from the noninfected patients were indistinguishable. Figure 6 shows that the four patients who were not infected with *H. pylori* apparently had been infected with the coccoid organism as judged by the strong serum IgG response to the coccoid antigens. These observations imply that the coccoid organism might be a causative agent of gastric ailments in some patients.

Initial taxonomic classification based on fatty acid composition and biochemical analysis. Our preliminary classification of strain SL100 shows similarity to *S. cohnii* based on fatty acid analysis with the MIDI/Hewlett-Packard microbial identification system and similarity to *S. xylosus* based on metabolic characterization with the Biolog identification system. These two species of *Staphylococcus* are closely related to each other based on nucleic acid hybridization analyses and have been implicated as potential human and animal pathogens (10). Further studies will be required for additional classification of strain SL100 and the other representative clinical isolates. Classification attempts involving rRNA techniques have not been carried out, since the organism cannot be routinely lysed by various techniques (sonication, French press, lysozyme, etc.) alone or in combination.

DISCUSSION

In industrialized Western countries, *H. pylori* is now recognized as the causative agent of most cases of gastritis and of gastric and duodenal ulcers (11). It is not clear if *H. pylori* is also the primary cause of gastroduodenal ailments in the developing nations. Strain SL100 and other isolates with similar coccoid forms were obtained from patients admitted to St. Vincent Hospital, a church-supported hospital in Suwon, Korea. This hospital provides low-cost health care to patients from a lower income group who live in neighborhoods more likely to be characterized by crowding and relatively poor sanitary conditions. It is not clear why others have not previously reported the isolation of this organism. We speculate that this organism may be more prevalent among Asians or in developing countries. The patients in this study were children during the 1950s and 1960s, when more people in Korea experienced poverty and less improved sanitary conditions. In future studies, we wish to examine the relative frequencies of isolation of coccoid forms compared to *H. pylori* and the presence of antibodies to the coccoid form and *H. pylori* in patients from various socioeconomic groups. We do not know if others have isolated this organism from biopsy material and considered it to be a contaminant. In this context, it is important to note that the urease activity of this isolate is significantly higher than that of the typical urease-positive *Staphylococcus* species. In the standard urease test using phenol red, the pH takes 5 to 30 s to change in strain SL100, while it takes 18 to 20 h in a urease-positive *S. aureus* isolate under comparable conditions.

We report here that some of the patients in this study were infected by a coccoid organism. These observations and the effects of strain SL100 following oral administration to neonatal pigs support the notion that this coccoid organism is pathogenic for humans and pigs. It is of interest that strain SL100 binds to mucin only under acidic conditions (Fig. 2). This acid-dependent binding property would ensure that the organism colonizes only the gastric mucosa and not other organs. At pH 3.5, strain SL100 binds to the porcine gastric antrum, but under the same conditions, *S. aureus* fails to bind (Fig. 4). This indicates that typical *S. aureus* strains would not normally be expected to colonize the gastric antrum and implies that strain SL100 is not a transient resident of the gastric antrum.

Strain SL100 and other such coccoid isolates assume a variety of microscopic morphologies including spheres, dented and wrinkled spheres, and other morphologic variants. While several coccoid variants of *H. pylori* have been reported, including some similar in appearance to strain SL100, these *H. pylori* coccoid forms have been reported to be noncultivatable and noninfectious (4).

During the course of these studies, one of us (C.K.) complained of severe stomach pain, and we suspected infection by the coccoid isolate. We examined his serum for the presence of antibodies against the coccoid organism, and as controls we examined the sera of five individuals without stomach pain. The serum of C.K. but not the sera of the other five individuals reacted with several microbial proteins from the coccoid iso-

late in a Western blot. The serum of C.K. did react in control experiments with proteins extracted from *Escherichia coli*, *Streptococcus faecalis*, and *Micrococcus luteus*, suggesting that individuals at this institute were infected by common human commensals. If the coccoid isolate were a common human commensal (e.g., *S. epidermidis*), all six individuals would be expected to have antibodies against this organism. In addition, we collected mouth washings from these six individuals by vigorous tooth brushing. These samples of oral flora were collected on Millipore filters and placed on blood agar plates. Profuse growth of the oral flora resulted, but none of the organisms from any of these individuals, including C.K., has the strong urease activity typical of the coccoid isolates obtained by biopsy of the patients with gastric symptoms in this study.

ACKNOWLEDGMENTS

We thank David H. Calhoun for comments on the manuscript and gratefully acknowledge the superb technical assistance of Kumju Yu.

REFERENCES

1. **Bode, G., P. Malfertheiner, and H. Ditschuneit.** 1988. Pathogenic implication of ultrastructural findings in *Campylobacter pylori* related gastroduodenal disease. Scand. J. Gastroenterol. **23**(Suppl. 142)**:**25–39.
2. **Boren, T., and P. Falk.** 1994. *Helicobacter pylori* binds to blood group antigens. Sci. Med. (Sci. Am.) **1994**(Sept/Oct)**:**28–37.
3. **Eaton, K. A., and S. Krakowka.** 1994. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect. Immun. **62:**3604–3607.
4. **Eaton, K. A., C. E. Catrenich, K. M. Makin, and S. Krakowka.** 1995. Virulence of coccoid and bacillary forms of *Helicobacter pylori* in gnotobiotic piglets. J. Infect. Dis. **171:**459–462.
5. **Engstrand, L., K. Rosberg, R. Hübinette, T. Berglindh, W. Rolfsen, and S. Gustavsson.** 1992. Topographic mapping of *Helicobacter pylori* colonization in long-term-infected pigs. Infect. Immun. **60:**653–656.
6. **Evans, D. G., D. J. Evans, Jr., and D. Y. Graham.** 1992. Adherence and internalization of *Helicobacter pylori* by HEp-2 cells. Gastroenterology **102:** 1557–1567.
7. **Ferrero, R. L., and A. Lee.** 1991. The importance of urease in acid protection for the gastric-colonizing bacteria *Helicobacter pylori* and *Helicobacter felis* sp. nov. Microbiol. Ecol. Health Dis. **4:**121–134.
8. **Haas, A., and W. Goebel.** 1992. Microbial strategies to prevent oxygen-dependent killing by phagocytes. Free Radical Res. Commun. **16:**137–157.
9. **Hansing, R. L., H. D'Amico, M. Levy, and R. A. Guillan.** 1992. Prediction of *Helicobacter pylori* in gastric specimens by inflammatory and morphological histological evaluation. Gastroenterology **87:**1125–1131.
10. **Kloos, W. E.** 1980. Natural populations of the genus *Staphylococcus*. Annu. Rev. Microbiol. **34:**559–592.
11. **Marshall, B. J.** 1994. *Helicobacter pylori*. Am. J. Gastroenterol. **89**(Suppl.)**:** 116–128.
12. **Morrison, M.** 1980. Lactoperoxidase-catalyzed iodination as a tool for investigation of proteins. Methods Enzymol. **70:**214–220.
13. **Nakada, K., T. Mizuta, Y. Tonokatu, Y. Fukuda, H. Okamura, T. Hayashi, T. Shimoyama, and T. Tamura.** 1992. Monoclonal antibodies against the native urease of *Helicobacter pylori*: synergistic inhibition of urease activity by monoclonal antibody combinations. Infect. Immun. **60:**4826–4831.
14. **Nielsen, H., and L. P. Anderson.** 1992. Activation of human phagocyte oxidative metabolism by *Helicobacter pylori*. Gastroenterology **103:**1747–1753.
15. **Olivieri, R., M. Bugnoli, L. D. Armellini, S. Bianciardi, R. Rappuoli, P. F. Bayelli, Abate, E. Esposito, L. de Gregorio, J. Aziz, C. Basagni, and N. Figura.** 1993. Growth of *Helicobacter pylori* in media containing cyclodextrins. J. Clin. Microbiol. **31:**160–162.
16. **Pérez-Pérez, G. I., A. Z. Olivares, T. L. Covers, and M. J. Blaser.** 1992. Characteristics of *Helicobacter pylori* variants selected for urease deficiency. Infect. Immun. **60:**3658–3663.
17. **Rudmann, D. G., K. A. Eaton, and S. Krakowka.** 1992. Ultrastructural study of *Helicobacter pylori* adherence properties in gnotobiotic piglets. Infect. Immun. **60:**2121–2124.
18. **Talley, N. J., J. E. Ormand, C. A. Frie, and A. R. Zinsmeister.** 1992. Stability of pH gradients in vivo across the stomach in *Helicobacter pylori* gastritis, dyspepsia, and health. Am. J. Gastroenterol. **87:**590–594.

Editor: V. A. Fischetti