Low Antigenicity of the Polysaccharide Region of *Helicobacter pylori* Lipopolysaccharides Derived from Tumors of Patients with Gastric Cancer

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We have examined the antibody response to *Helicobacter pylori* lipopolysaccharide (LPS) during natural infection in humans. The sera of over 70% of *H. pylori*-infected individuals were found to contain immunoglobulin G antibodies against the LPS fractions isolated from smooth strains of *H. pylori* but not against those derived from rough strains, as determined by enzyme-linked immunosorbent assay. These results taken together with the immunoblot data indicated that the polysaccharide region of *H. pylori* LPS is antigenic in humans. However, the antigenicity of the polysaccharide varied, depending on the strain. We found that smooth *H. pylori* strains isolated from the tumors of patients with gastric cancer showed significantly lower antigenicity than smooth strains derived from patients with chronic gastritis and gastric and duodenal ulcers. The results suggest that the levels of antigenicity of the polysaccharide region of *H. pylori* LPS in humans correlate with the nature of the gastrointestinal diseases and that they allow a particular distinction to be made between gastric cancer and other gastrointestinal diseases, especially chronic gastritis.

**MATERIALS AND METHODS**

**Bacterial strains.** Clinical strains of *H. pylori* were isolated from the biopsy specimens of lesions obtained from patients with chronic gastritis (strains CG1 to -19), gastric ulcers (strains OU1 to -18), duodenal ulcers (strains DU1 to -9), and gastric cancer (from tumors and nontumorous tissues, strains CA1 to -10 and CA1N to -10N, respectively) in the Sapporo Medical University Hospital (Sapporo, Japan). *H. pylori* 43504r was a spontaneous rough mutant derived from ATCC 43504, which was purchased from the American Type Culture Collection (Rockville, Md.). After three to five laboratory subcultures, these bacteria were grown on brain heart infusion agar plates supplemented with 10% (vol/vol) horse blood at 37°C for 5 days in microaerophilic conditions by using the Gas Pack system (BBL, Cockeysville, Md.) without a catalyst. The organisms were collected, washed with phosphate-buffered saline three times, and lyophilized.

**Human sera.** The sera of 25 patients with chronic gastritis (14 sera), gastric ulcers (4 sera), duodenal ulcers (4 sera), and gastric cancer (3 sera) were collected independently of the isolation of *H. pylori* strains used in this study. The sera of 83 healthy adult volunteers were obtained from Akita University Hospital. The status of *H. pylori* infection of each individual was monitored by the *H. pylori* antibody determiner enzyme immunoassay kit (high-molecular-weight cell-associated protein [HM-CAP] kit) (Kyowa Medics, Tokyo, Japan).

**SDS-PAGE and immunoblotting.** LPS fractions were prepared by protease K (Merck, Darmstadt, Germany) treatment. Briefly, the lyophilized cells were dissolved in the sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (9) at a concentration of 2 mg/ml and incubated at 100°C for 10 min. Then 200 μl of a solution of protease K (2.5 mg/ml) per ml was added, and the mixture was incubated at 37°C overnight followed by incubation at 65°C for 2 h. The LPS fraction was applied to a 12.5% (wt/vol) polyacrylamide gel slab and electrophoresed. The LPS profile on the gel was developed by silver staining according to the method of Hitchcock and Brown (7). Immunoblotting was accomplished by the method described previously (1). After the LPS fraction was transferred from the gel to a GVHP filter (Nikon Millipore, Yonezawa, Japan), the filter was incubated with human sera (1:100 dilution) as the first antibody. A horseradish peroxidase-conjugated goat anti-human immunoglobulin G (IgG) antibody (Dako, Copenhagen, Denmark) and 3,3′-diaminobenzidine (Nakarai Tesque, Kyoto, Japan) were used as the second antibody and the substrate, respectively.

**Enzyme-linked immunosorbent assay (ELISA).** The proteinase K-treated cells described above were used as a source of LPS fractions. *Salmonella minnesota* R595 (Re mutant) LPS was purchased from Sigma (St. Louis, Mo.). The LPS fraction was diluted 1:50 or diluted at a concentration of 10 μg/ml with 50 mM sodium carbonate buffer (pH 9.6). Then the diluent was dispensed in a 96-well polystyrene microtiter plate (Becton Dickinson, Oxnard, Calif.) and incubated at 4°C overnight. The plate was blocked with 1% (wt/vol) bovine serum albumin by incubation at 37°C for 2 h. Human serum was diluted 1:3,000 with phosphate-buffered saline containing 0.05% Tween 20 and dispensed to the microplate. Horseradish peroxidase-conjugated goat F(ab′)2 anti-human IgG antibodies (BioSource International, Camarillo, Calif.) and TMB peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) were used as the second antibody and the substrate, respectively. Absorbance at 450 nm (A450) was measured after the reaction was terminated with 1 M phosphoric acid. Binding activity was expressed as the mean A450 value of triplicate measurements.

**Statistical analysis.** The statistical significance of the differences between the mean values of binding activity in ELISA was carried out by the Student t test.

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RESULTS

Antibody response to *H. pylori* LPS. We chose 11 strains of *H. pylori* for a detailed characterization. The LPS profiles on silver-stained SDS-polyacrylamide gels are shown in Fig. 1A. Among them, 43504-r and CG10 were rough strains and thus did not display polysaccharide chains. All the other strains were smooth strains, and their polysaccharide chains appeared as a series of ladder bands on the silver-stained gels.

First we examined the status of *H. pylori* infection of each individual with the conventional serum diagnosis kit HM-CAP. The sera of 24 of 25 patients and 21 of 83 healthy volunteers were found to be positive for *H. pylori* infection. The antibody response to LPS was determined by ELISA with the LPS fraction isolated from proteinase K-treated cells as the immobilized antigen. From the results of ELISA at various dilutions of sera, 3,000-fold dilution was found to be suitable for detecting the titer of antibody specific to *H. pylori* LPS and eliminating nonspecific binding (data not shown). More than 70% of the sera of *H. pylori*-infected individuals contained IgG against *H. pylori* LPSs as determined by ELISA. Sera were obtained from patients with gastroduodenal diseases (A), healthy individuals with *H. pylori* infections (B), and healthy individuals without *H. pylori* infections (C). Human sera were diluted 3,000-fold and applied to a microplate coated with *H. pylori* LPS, and the bound IgG was measured. Serum titer is expressed as the mean value of the ELISA readings (A_{450}) of triplicate measurements. HM-CAP was from the HM-CAP ELISA kit. Re, LPS from *S. minnesota* R595 (Re mutant).

Comparison of antigenicities of LPSs of *H. pylori* strains derived from various sources. The antigenicities of LPSs from 69 *H. pylori* strains were analyzed by using 10 randomly selected high-titer human sera as probes (Fig. 2). The LPS of *H. pylori* showed varying antigenicities, depending on the strain. Rough strains showed low antigenicity regardless of their clinical sources. Eight of nine smooth strains derived from tumors of gastric cancer patients showed low antigenicities. The low antigenicities of strains derived from these tumors was a significant difference from those of strains isolated from other gastroduodenal diseases, especially chronic gastritis. Furthermore, we compared the antigenicities of paired strains derived from the tumors and nontumorous tissues of individual gastric cancer patients (Fig. 4). In 2 out of 10 cases, isolated strains derived from nontumorous tissues were highly antigenic whereas strains derived from tumors showed low antigenicities.

DISCUSSION

In this study, we have demonstrated that the polysaccharide region of *H. pylori* LPS is a potent immunogen in humans during natural infections. The LPSs from the smooth strains,
which carry the O polysaccharide chains, could be classified into two groups, high antigenicity and low antigenicity. We focused our attention on the relationship between the antigenicity and the nature of the clinical sources of H. pylori. The antigenicities of the H. pylori strains isolated from patients with gastric tumors were significantly lower than those of strains isolated from patients with other gastroduodenal diseases. The low level of antigenicity may be related to the progression of the disease. This may be particularly relevant to gastric cancer compared to, for instance, chronic gastritis. It should be emphasized, however, that the low antigenicity was not due to the lack of the O polysaccharide, because the low-antigenicity strains expressed normal levels of polysaccharide chains.

Our study raises a number of questions Why do strains isolated from gastric tumors express a low-immunogenic LPS? One possibility is that low-antigenicity LPS is tumorigenic. This hypothesis suggests that tumorigenic strains of H. pylori may exist. However, there is no data in the literature in support of the tumorgenicities of individual H. pylori strains. Another possibility is that H. pylori cells carrying a low-antigenicity LPS are selected for during infection of the host. Changes in the structure of the cell surface antigens during infection would allow H. pylori to escape from the host immune system. This would be applicable to gastric cancer, which is regarded as the later stage of a series of inflammatory reactions (5). The gastric cancer patients would have been exposed to the inflammatory stimuli caused by H. pylori infection longer than the patients with other gastroduodenal diseases. In any case, it will be necessary to conduct longitudinal studies of the antigenic changes of H. pylori cells during the course of infection. Our finding that two strains isolated from the tumors of patients have low antigenicities compared to those of strains isolated from the nontumorous tissues of the same patient (Fig. 4) deserves further investigation. It would be interesting to know, for example, whether or not these strains are derived from the same H. pylori organism.

Another question is what structures of H. pylori LPS represent the antigenic determinants. Data from immunoblotting (Fig. 1B to F) indicates that the determinants are located on the polysaccharide region of the LPS preparation. Recently, Lewis antigen structures were identified as being part of the polysaccharides of many H. pylori LPSs. Appelmelk et al. suggested that the Lewis antigen, especially Lewis α, structure is an antigenic epitope in mammals, including humans (2), and Wirth et al. indicated that the distribution of Lewis α and Lewis α structures was related to a gene, cagA, associated with pathogenicity. However, we have evidence to show that the distributions of antigenic determinants and of Lewis α antigens in H. pylori LPSs may not be so directly related. In fact, some strains from gastric tumors, which show low antigenicities, expressed Lewis α antigens and some highly antigenic LPSs were found not to contain Lewis α antigens (1a, 18). At least some antigenic determinants for humans with natural infections seem to be immunologically distinct from Lewis structures, although we do not question the fact that the determinants located on the polysaccharide chains contain the Lewis antigens.

H. pylori is recognized as a category 1 (definite) human carcinogen by the World Health Organization and the International Agency for Research on Cancer (10). However, there is little experimental evidence showing cancer development induced by H. pylori infection. The observations in the present paper suggest that H. pylori strains isolated from tumors are different from those from other sources. Further study of this phenomenon should provide useful information concerning H. pylori pathogenicity with respect to gastric cancer. Moreover, the method described here could eventually be applied to the screening of individuals who are at the highest risk of developing gastric cancer induced by H. pylori infection.

REFERENCES


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