Clinical Relevance of the babA2 Genotype of Helicobacter pylori in Japanese Clinical Isolates

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Helicobacter pylori infection in the stomach activates a mucosal inflammatory response and leads to diverse clinical outcomes in humans (3). Most infected persons are asymptomatic with histologic gastritis (22), or they may develop a gastric or duodenal ulcer (1), adenocarcinoma (13), or mucosa-associated lymphoid tissue (MALT) lymphoma (27, 32) of the stomach. Numerous studies indicate that both bacterial and host factors may be important in leading to particular clinical and pathological sequelae of the infection. Among the bacterial factors, the role of the ability to adhere to epithelial cells is crucial in the initiation of a gastric inflammatory response (17, 24, 28). The blood group antigen-binding adhesin BabA has been shown to mediate adherence of H. pylori to human Lewis b (a-1,3/4-difucosylated) blood group antigens on gastric epithelial cells (5). In vitro adherence assays revealed that H. pylori bound in a lineage-specific manner to gastric surface mucous cells mediated by fucosylated blood group antigens (11). Furthermore, a study using transgenic mice expressing the human Lewis b epitope in gastric epithelial cells indicated that Lewis b antigens function as receptors for an H. pylori adhesin and mediate its attachment to gastric pit and surface mucous cells (12). The attachment of H. pylori to gastric epithelial cells in such transgenic mice resulted in the development of chronic gastritis and gastric atrophy (15). In a recent study, the gene encoding BabA was cloned (and named babA2), thus allowing the identification of H. pylori strains harboring the babA2 genotype by PCR (18).

The cag pathogenicity island is one of the major virulence factors of H. pylori, and there is a high frequency of the presence of the cagA gene in the cag pathogenicity island in patients with duodenal ulcers (16), atrophic gastritis (21, 22), gastric carcinomas (4), and MALT lymphomas (9) in Western countries. However, in Japan H. pylori strains harboring the cagA gene have not been related to clinical outcomes because of the very high prevalence of strains harboring the cagA gene in Japanese clinical isolates (25, 29). A recent study has shown that the presence of the babA2 gene was significantly associated with duodenal ulcers and gastric carcinomas and with the presence of the cagA genotype in a Western population (14).

The clinical relevance of the H. pylori babA2 genotype has not yet been determined in a large series of clinical isolates in Japan. Therefore, we investigated the presence of babA2 and cagA in Japanese clinical H. pylori isolates and their correlation with clinical outcomes (nonulcer dyspepsia, duodenal ulcer, gastric ulcer, gastric adenocarcinoma, and MALT lymphoma).

MATERIALS AND METHODS

Subjects. One hundred seventy-nine H. pylori strains were obtained from antral and corpus biopsies from Japanese patients between 1997 and 1999 at Hokkaido University Hospital, Sapporo, Japan. None of the patients had received nonsteroidal anti-inflammatory drugs or antibiotics within the previous 3 months. The patient population consisted of 179 patients (117 men and 62 women) with a mean age of 48.6 years (range, 21 to 74 years). The patients were classified at the time of endoscopy into those having gastric ulcers (n = 45), duodenal ulcers (n = 41), gastric adenocarcinomas (n = 40), MALT lymphomas (n = 11), or no evidence of mucosal ulceration but with chronic gastritis (non-ulcer dyspepsia) (n = 42). The classification of patients was based on the results of endoscopic and histological examinations.

Bacterial strains. Biopsy specimens were cultured on H. pylori-selective agar plates (Eiken Chemical Co., Ltd., Tokyo, Japan) under microaerophilic conditions (5% O2, 10% CO2, 85% N2 at 37°C; AaeoPack Systems, Mitsubishi Gas Chemical, Osaka, Japan) for up to 5 days. The organisms were identified as H. pylori by Gram staining, colony morphology, and positive oxidase, catalase, and urease reactions. A single colony on the agar was collected and cultured again under the same microaerophilic conditions in brain heart infusion broth (Nissui, Osaka, Japan) containing 5% (vol/vol) horse serum for up to 5 days. Aliquots were stored at −80°C in 10% phosphate-buffered saline containing 20% (vol/vol) glycerol. After thawing of the aliquots of the frozen culture, bacterial suspensions were cultured at 37°C in brain heart infusion broth containing 5% fetal calf serum (GIBCO BRL, Rockville, Md.) under microaerobic conditions as de-
scribed above on a gyratory shaker at 160 rpm for 24 to 36 h to the plateau phase. The bacterial suspensions were centrifuged at 2,000 × g for 5 min, and the bacterial pellets were used for genomic DNA extraction. Genomic DNA was extracted by using a SepaGene kit (Nippon Gene, Toyama, Japan) according to the manufacturer’s instructions.

PCR. PCR was performed according to a previously reported method (2). An aliquot (0.5 μl) of Tag DNA polymerase and deoxynucleoside triphosphates (Takara Shuzou Co., Ltd., Shiga, Japan) was mixed with 1 μl of a genomic DNA sample of each strain and primer. The babA2 primers were designed on the basis of the recently published signal sequence of the babA2 gene (14). The primers used were babA2F (5’-AATCCAAAAGGAAAAAGTGATGAA3’-3”) and babA2R (5’-TGTTAGTGTATTTGGTAGGACA-3’) for babA2 amplification, ureAF (5’-GCCAATGTTAATTAGTT-3’) and ureAR (5’-CTCCTTTAATGTGTTACACAG-3’) for ureA amplification, and cagAF (5’-GCGCTATATCCTATCTTTC-3’) and cagAR (5’-GCCCTGAAGTTGATGGAACACCC-3’) for cagA amplification. These base sequences corresponded to the nucleotide sequences of strain NTCC 11638 or strain J99 (31). PCR was performed using a thermal cycler (Takara Shuzou) under the following conditions: an initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. PCR amplification of the H. pylori ureA gene was performed as a positive control.

Southern blot analysis. Ten micrograms of genomic DNA of H. pylori was digested by restriction enzyme MboII (New England BioLabs, Beverly, Mass.), electrophoresed on a 1% agarose gel, and then transferred onto a nylon membrane. The cagA probe was made from the same primers as those described above and labeled with digoxigenin (DIG) using a PCR DIG probe synthesis kit (Boehringer Mannheim GmbH, Mannheim, Germany). The cagA probe was located in the upstream region of the total cagA gene (381 of 4,043 bp). These base sequences corresponded to the nucleotide sequences of the cagA gene of strain NTCC 11638. The membrane was hybridized with the labeled probe for 20 h at 42°C in DIG Easy Hyb (Boehringer Mannheim GmbH). After being washed sequentially in 2× SSC–0.1% sodium dodecyl sulfate (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.2× SSC–0.1% sodium dodecyl sulfate, the detection of the membrane was done by using a DIG nucleic acid detection kit (Boehringer Mannheim GmbH) according to the manufacturer’s instructions.

Statistical analysis. Fisher’s exact test was used for the analysis of categorical data (Table 1). Analyses were done using Stat View Software, version 4.5 (SAS Institute Inc., Cary, N.C.). A P value of <0.05 was accepted as statistically significant.

RESULTS

PCR and Southern blot analysis. The babA2 genotype was detected in 152 of the 179 isolates (84.9%) by PCR. The cagA genotype was detected in 172 of the 179 isolates (96.1%) by PCR and Southern blot analysis. The babA2-positive strains showed the presence of cagA (146 of 152 isolates [96.7%]). Twenty-six of the 27 babA2-negative strains showed the presence of cagA (96.3%). Only one of the 27 babA2-negative strains (3.7%) did not show the presence of cagA. There was no correlation between the babA2 genotype and the cagA genotype.

Relationship between prevalence of cagA and babA2 genotypes and clinical outcome. Table 1 shows the relationship between the prevalence of the cagA and babA2 genotypes and clinical outcome. The overall prevalence of the babA2 genotype was 84.9% (152 of 179 isolates). No significant correlation was obtained between the babA2 genotype and clinical outcome.

The overall prevalence of the cagA genotype was 96.1% (172 of 179 isolates). The presence of the cagA genotype was not correlated with any clinical outcomes (Table 1).

DISCUSSION

The adherence of H. pylori to epithelial cells is a crucial factor in the specific tropism and pathogenicity of the organism in the stomach (4, 16, 17, 24, 28). Biochemical studies have identified a protein from H. pylori, an adhesin named BabA, that allows binding to the blood group antigen Lewis b present on the surface of gastric epithelial cells (6). Two corresponding genes encoding BabA have been cloned and named babA1 and babA2 (18). Only the babA2 gene is functionally active (18). These two genes have almost complete sequence homology, with the exception of a translational initiation codon in the signal peptide sequence found only in babA2 (18). A recent study has shown that a mismatch PCR method that amplifies the signal peptide sequence is an efficient method for selective identification of the presence of the babA2 gene (14). The distribution of the presence of the babA2 genotype in Western countries has been shown to be about 66 to 72% in recent studies (14, 18). One of these studies has suggested that the presence of babA2, vacAs1, and cagA (“triple-positive” strains) showed a highly significant correlation to the prevalence of duodenal ulcers and gastric adenocarcinomas (14). Although a previous study in Japan showed that almost all Japanese strains (97.7%) harbored the vacAs1 genotype (19), we tested for the presence of the vacAs1 genotype by PCR (19). In our study, it was confirmed that 172 of 179 strains (96.1%) showed the vacAs1 genotype. In addition, there was no correlation between the babA2 and vacAs1 genotypes (data not shown).

The present study suggested that the prevalence of the babA2 genotype is higher in Japan than in Western countries and that there is not a significant correlation between the babA2 genotype and clinical outcome in Japan. These results are not in accordance with those of a recent study in a Western population (14). In Japan, only a few patients infected with babA2-, cagA-, or vacAs1-positive strains will suffer from peptic ulcers or gastric adenocarcinomas. It is therefore difficult to explain the different clinical outcomes from virulence factors only, such as babA2, cagA, and vacAs1 of H. pylori.

We tested the adherence abilities of babA2-negative strains compared with those of babA2-positive strains in an in vitro study in which a flow-cytometric assay was performed by using Lewis b-positive gastric epithelial cells (KATOIII cells). Our findings showed that the babA2-negative strains adhered more strongly than did the babA2-negative strains but that the babA2-negative strains adhered weakly to the cells (data not shown). It is still a fact that both the babA2-positive strains and the babA2-negative strains colonize in the stomach and that there are adherence factors other than the babA2 gene. We therefore investigated the presence of the hpaA gene, one of the bacterial adhesins (8, 10, 17, 23, 26), in babA2-negative strains by PCR. The hpaA gene was found to be present in all
babA2-negative strains (data not shown). We speculate that the babA2-negative strains can colonize in the human stomach because of the presence of other bacterial adhesins.

An explanation of the different clinical outcomes from the host factors, including histopathology of Lewis b expression in gastric tissue, may be possible, but a previous study in Japan showed that the majority of gastric biopsies from patients (95% of cases of normal foveolar epithelium, 75% of intestinal metaplasias, and 75% of intestinal types of gastric cancer) expressed Lewis b (20), and other previous studies in Western countries showed that the expression of the Lewis b antigen on primary gastric cells was about 95% (7, 30). One of those studies demonstrated that adherence of H. pylori to gastric epithelial cells was not dependent on the expression of either Lewis a or Lewis b on primary cells isolated from the biopsy and that incubation of primary gastric cells with monoclonal antibodies to either Lewis a or Lewis b had no effect on H. pylori binding (7). Thus, it seems to be difficult to explain the different clinical outcomes in Japan in terms of host factors such as the expression of Lewis b antigen on gastric epithelial cells and the presence of BabA.

In conclusion, our current data do not support the hypothesis that the virulence factors of H. pylori, BabA and CagA, are strongly associated with peptic ulcer disease and gastric adenocarcinoma in Western countries. We speculate that the prevalences of babA2 and cagA genotypes in Japan are much higher than those in Western countries.

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