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A *Helicobacter pylori* Restriction Endonuclease-replacing Gene, *hrgA*, Is Associated with Gastric Cancer in Asian Strains[1]

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ABSTRACT

The sensitivity of *Helicobacter pylori* chromosomal DNA to *Mbo*I digestion was investigated in 208 strains from several continents. Only 11 (5%) of strains were sensitive to *Mbo*I, and it was hypothesized that *Hpy*III, a type II restriction/modification enzyme with sequence homology to *Mbo*I, mediated the protection. This was confirmed by PCR analysis of the gene locus of *hpy*III, normally composed of *hpy*IIIR and *hpy*IIIM. In all but one strain sensitive to *Mbo*I, no PCR product of *hpy*IIIR was obtained. In contrast, all strains yielded a product for *hpy*IIIM, independent of *Mbo*I phenotype. Further examination of the *hpy*III locus in strains lacking a *hpy*IIIR PCR product identified a novel gene, *hrgA*, upstream of *hpy*IIIM. All 208 strains examined had either *hpy*IIIR or *hrgA*, but not both, upstream of *hpy*IIIM. Although *hrgA* has homology with a *Campylobacter jejuni* gene (*Cj1602*), its function is not known. In Western countries, *hrgA* was more prevalent (53%) than in Asia (25%; $P < 0.0001$, χ^2). In Asia, *hrgA* was more prevalent among gastric cancer patients (18 of 43; 42%) than among noncancer patients (16 of 95; 17%; $P = 0.001$, χ^2). All 143 Asian strains tested were *cagA*$^+$, but among Western strains, *hrgA* was more prevalent in *cagA*$^+$ strains (26 of 42; 62%) than in *cagA*$^-$ strains (9 of 23; 39%; $P = 0.04$, χ^2). In coculture with epithelial cells, *hpy*IIIR and *hrgA* strains did not show any significant differences in interleukin-8 induction and apoptosis. Although a direct function for *hrgA* in virulence could not be demonstrated, our data indicate that *hrgA* is a strain-specific gene that might be associated with gastric cancer among *H. pylori* isolates from Asian patients.

INTRODUCTION

Helicobacter pylori are Gram-negative bacteria that colonize the human stomach and whose presence affects the risk of upper gastrointestinal tract diseases, including gastric cancer (1). Several strain-specific factors have been identified that potentially are markers for the differential risk associated with *H. pylori* colonization; at present, *cagA*, a marker of the *cag* pathogenicity island, has the strongest predictive value (2–6). However, in East Asia, most strains are *cagA*$^+$ regardless of clinical outcome (7–9). Thus, identification of bacterial factors involved in or serving as markers for the progression to ulceration or to gastric cancer remains desirable. During our study of R-M[3] systems in *H. pylori*, we unexpectedly discovered such a potential marker that among Asian patients with *cagA*$^+$ strains identifies those associated with gastric cancer.

H. pylori strains are highly heterogeneous in the number and nature of the type II R-M systems they carry (10–15). Type II R-M systems comprise two enzymes encoded by paired genes, a restriction endonuclease that cleaves DNA within a specific 4–8-bp sequence and a methyltransferase that specifically methylates the DNA at adenine or cytosine residues within the same sequence and thus protects the sequence from cleavage (16–18). *H. pylori* DNA is highly methylated at both adenine and cytosine residues (10), consistent with genomic sequence analyses that predicted 14 and 15 potential R-M systems for *H. pylori* strains 26695 and J99, respectively (11, 12). The *hpy*III R-M gene locus (13–15) is homologous to the *Mbo*I R-M system of *Moraxella bovis* (19), which recognizes the DNA sequence GATC, and the same recognition sequence has been confirmed for *hpy*III (13, 14).

In a previous study (20), we showed that all *H. pylori* strains examined were resistant to *Nla*III and that most (95%) were also resistant to *Mbo*I. *Nla*III is homologous to *hpy*IR, which has been called *iceA1*, and *Mbo*I is homologous to *hpy*IIIR. In some *H. pylori* strains, *iceA2* replaces *iceA1*, and strains with *iceA1* have been found to be more highly associated with peptic ulcer disease (21, 22) and gastric cancer (23) than those with *iceA2*. These associations prompted us to study the *hpy*III R-M system in more detail to determine whether there was a similar correlation with pathogenic outcomes. We assayed a collection of strains for susceptibility to *Mbo*I. When a small minority of strains was found to be sensitive to this restriction enzyme, they were examined to detect polymorphisms in their *hpy*III locus. These analyses identified a new gene, *hrgA*, that had replaced *hpy*IIIR in most *Mbo*I-sensitive strains. For most strains investigated, the patient's clinical outcome was known, allowing investigation of correlations with *hrgA/hpy*IIIR status. Our findings suggest that *hrgA* can potentially be used as a marker for virulence in the presence of *cagA*.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. A total of 208 clinical isolates from different parts of the world (Table 1) were from patients with duodenal ulcers ($n = 55$), gastric ulcers ($n = 42$), gastric cancer ($n = 43$), and nonulcer dyspepsia ($n = 62$). The three Colombian strains were isolated from Hispanic persons and categorized as Western strains. For six patients (five from Thailand, one from the United States), clinical data were not available. The strains were obtained from 208 unique patients. At the time of endoscopy, two biopsy specimens were obtained from the greater curvature of the antrum, between 2 and 5 cm from the pylorus. To isolate *H. pylori*, biopsies were homogenized in 250 μl of saline, and 50 μl were plated onto trypticase soy agar with 5% sheep blood (BBL) and incubated for up to 9 days under microaerobic conditions. Single colonies were collected, and bacteria were identified as *H. pylori* by Gram's stain morphology as well as by urease and oxidase activity. All isolates were characterized by their *cagA* status (positive, $n = 185$; negative, $n = 23$). Strain JP26, from which *hrgA* was originally isolated and sequenced, is a *H. pylori* strain obtained from a gastric cancer patient in Japan.

DNA and Protein Techniques. Standard molecular techniques were used (24). *H. pylori* chromosomal DNA was prepared from cells of each strain after 48 h of growth on two agar plates as described (25). PCR reactions were performed in reaction volumes of 50 μl containing 0.5 units of Taq polymerase (Qiagen), 1.5 mM $MgCl_2$, and 200 ng of each primer. The PCR protocol (30

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[3] The abbreviations used are: R-M, restriction-modification; IL, interleukin; FBS, fetal bovine serum.