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Characterization of Chromosomal DNA Profiles from *Helicobacter pylori* Strains Isolated from Sequential Gastric Biopsy Specimens

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The restriction endonuclease profiles of DNAs from *Helicobacter pylori* strains isolated from 20 patients in two or more consecutive biopsy specimens over a period of up to 2 years were analyzed by pulsed-field gel electrophoresis with *Not*I and *Nru*I. *H. pylori* strains possess a high degree of genomic diversity which was not observed to occur in vivo, and attempts to observe it in vitro were not successful.

Strains of *Helicobacter pylori* have been associated with gastritis (1, 5) and peptic ulcer disease (4). Recent reports have also suggested a role for these organisms in the development of gastric carcinoma (10). *H. pylori* strains survive in the acidic environment of the stomach by a variety of mechanisms such as the production of large amounts of urease (7, 12) and as a result of their possession of bipolar flagella that aid their movement across the mucous layer (8). Other virulence factors that have been suggested to play a role in the production of disease include the 87-kDa cytotoxin that causes the formation of vacuoles in eukaryotic epithelial cells (3, 16) and a cytotoxin-associated antigen of 128 kDa (2).

We have reported previously that strains of *H. pylori* possess a great deal of diversity in their genomic DNA restriction profiles when analyzed by pulsed-field gel electrophoresis (PFGE) (14); however, the reasons for this diversity are unclear. The aims of the study described here were to compare DNA profiles between consecutive isolates of *H. pylori* and to determine if genetic rearrangements are occurring in vivo. We also examined the stabilities of these DNA profiles in vitro.

Strains of *H. pylori* were isolated from gastric antral biopsy specimens from 20 patients examined at the Division of Gastroenterology at the University of Alberta Hospital (Table 1). The organisms were grown on plates of brain heart infusion agar (Unipath Limited, Nepean, Ontario, Canada) supplemented with 5% fetal calf serum (Gibmar Laboratories, Edmonton, Canada), 20 μg of vancomycin (Sigma Chemical Company, St. Louis, Mo.) per ml, and 15 μg of amphotericin B (Sigma) per ml as described previously (14). These strains were stored in a 1% peptone–25% glycerol solution (pH 7.0) at −90°C until they were needed. Six to 30 months after the initial isolation, *H. pylori* strains were reisolated from the same patients by the same procedures. From two patients, biopsy specimens were obtained on three consecutive occasions (Table 1).

Strains of *H. pylori* were identified by their translucent colony morphology, corkscrew-like motility on wet film preparations, negative Gram stain, and positive oxidase and urease reactions (6).

Genomic DNAs of *H. pylori* strains isolated from the 20 patients were prepared for PFGE as described previously (14). DNA inserts of 1 to 2 mm in thickness were digested with the restriction endonucleases *Not*I and *Nru*I, and DNA fragments were separated by using the contour-clamped homogeneous electric field electrophoresis system of PFGE (14). DNA patterns were visualized by staining the gels in a solution containing ethidium bromide and placing them on a UV light source. The gels were photographed by using a Pentax 35-mm camera and Kodak Tri-X Pan film as described previously (14). The DNA patterns of the *H. pylori* strains isolated from the same patients and from other patients were analyzed.

Information on *H. pylori* isolates including the symptoms that they caused, the date of isolation, and the numbers of *Not*I and *Nru*I restriction fragments generated from the DNA is provided in Table 1. DNAs from 15 of the *H. pylori* strains could be digested with the restriction endonuclease *Not*I. DNAs from five strains could not be digested with the restriction enzyme *Not*I, but DNAs from four of these strains could be digested with the restriction enzyme *Nru*I. Furthermore, the DNAs of seven strains could not be digested with *Nru*I, but the DNAs of six of these strains could be digested with *Not*I. DNA from each *H. pylori* strain that was digested gave a unique fingerprint. Strains isolated from the same patient at different intervals showed identical genomic DNA restriction profiles when the DNAs were analyzed by PFGE (Fig. 1).

No change in the restriction endonuclease profiles of 10 of these *H. pylori* strains was noted after 8 to 12 passages of the strains on culture medium. All strains retained their original restriction profiles.

To determine if any of the patients may have been colonized with more than one strain of *H. pylori*, 10 single colonies were selected from the initial isolation plate containing the specimen obtained from each of the 20 patients and were processed for PFGE as described above. The restriction endonuclease patterns of whole chromosomal DNAs of the different colonies isolated from the same plate after digestion with the restriction endonucleases *Not*I and *Nru*I were identical (data not shown). These data imply that these patients were colonized with a single *H. pylori* strain.

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TABLE 1. Restriction endonuclease profiles of *H. pylori* strains isolated from gastric biopsy specimens from patients with various symptoms

Patient no.	UA identification no. of *H. pylori* isolates[a]			No. of restriction enzyme fragments		Clinical diagnosis[b]
	A	B	C	*Not*I	*Nru*I	
1	831	945 (12)		>20	0[d]	GU + E
2	829	954 (22)		6	7	DU
3	903	946 (4)		7	3–6[c]	DU
4	942	987 (8)		4	6	DU + GU
5	877	975 (12)		5	6	GU + L
6	880	985 (12)		7	2	DU
7	939	974 (6)		0[d]	6	GU
8	797	940 (15)		7	0[d]	DU
9	878	1173 (20)		6	2	DU
10	982	1154 (6)		0[d]	4	DU
11	951	1152 (6)		1	0[d]	DU
12	944	992 (12)		6	0[d]	DU
13	915	1111 (6)		6	0[d]	DU + E
14	910	980 (12)		7	4	GU + D
15	891	993 (9)		0[d]	2	DU
16	890	1109 (14)		0[d]	0[d]	DU
17	804	988 (15)		6	0[d]	GU
18	828	1000 (21)		6	5	DU
19	799	1182 (12)		5	>20	DU + G
20	825	916 (10)	1109 (22)	0[d]	6	E

[a] The numbers represent strain numbers, and numbers in parentheses represent the period (in months) from the time of initial isolation. A, first isolate; B, second isolate; C, third isolate.

[b] A single biopsy specimen was obtained from each patient at each time point. All biopsy specimens showed evidence of gastritis by the criteria described by Whitehead et al. (17); also noted were duodenal ulcer (DU), gastric ulcer (GU), esophagitis (E), duodenitis (D), and lymphoma (L).

[c] Inconsistent digest patterns were obtained with this pair of strains, with *Nru*I showing three to six fragments.

[d] Absence of cutting sites.

The present study confirms that strains of *H. pylori* possess a great diversity at the genomic level (11, 14). This phenomenon was not noted in another species of *Helicobacter* (*H. mustelae*) (13), nor was it noted in the closely related *Campylobacter* species (15). DNA polymorphism was, however, previously noted in the strains of "*Deinococcus radiopugnans*" resistant to UV light and ionizing radiation. It was hypothesized, then, that this diversity may have been caused by a genomic rearrangement, but the reasons for this rearrangement could not be explained (9).

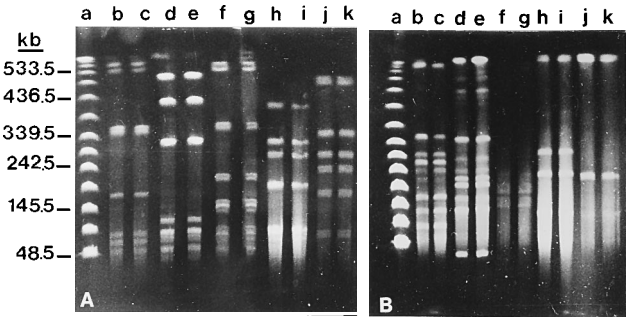

FIG. 1. PFGE of chromosomal DNAs of *H. pylori* strains isolated from patients at different intervals (Table 1) digested with the restriction endonucleases *Not*I (A) and *Nru*I (B). Phage λ DNA concatemers (48.5 kb) were used as size markers (lanes a). *H. pylori* strains are as follows: UA831 (lanes b), UA945 (lanes c), UA829 (lanes d), UA954 (lanes e), UA903 (lanes f), UA946 (lanes g), UA877 (lanes h), UA975 (lanes i), UA939 (lanes j), and UA974 (lanes k). Strains UA903 and UA946 gave three to six identical fragments when their DNAs were cut with *Nru*I; however, these were of inconsistent size.

It was suggested previously that the observed diversity was due to the exchange of genetic material between different strains of *H. pylori* as a result of the process of natural transformation in vivo (14). This process would be feasible when more than one strain is available in proximity. We did not isolate different strains of *H. pylori* from the same patient. As a result, this mechanism remains open to speculation. Passaging the strains up to 12 times on culture medium also did not lead to changes in the DNA profiles. The mechanisms and reasons for the observed diversity are still unclear. However, further studies with animal models might indicate the stage at which rearrangement of the restriction sites occurs. This could include ingestion of live *Helicobacter* strains with known restriction patterns and then reisolation of these organisms after a period of time to determine if the change in the restriction pattern occurs at the stage of introduction to a new host.

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