

Lewis Antigen Expression by *Helicobacter pylori* Strains Colonizing Different Regions of the Stomach of Individual Patients[∇]

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The diversity in the expression of Lewis antigens (Le) of 226 single colonies of *Helicobacter pylori* isolated from four regions of the stomach of eight adults is shown. Le^y was expressed more in strains colonizing antrum than in strains colonizing fundus, whereas Le^x was more common in fundus strains. *cagA*⁺ strains were more associated with Le-negative strains.

The *Helicobacter pylori* Lewis antigens (Le) mimic Lewis blood group antigens found on the surfaces of human gastric cells, providing the bacteria with a possible mechanism of immune evasion (5). This intimate interaction with the epithelia suggests that adaptation to specific regions of the stomach might occur. Most reports have studied *H. pylori* Le expression in isolates from the antrum and corpus exclusively (6–8, 10); the present study sought to characterize Le antigen expression in multiple colonies isolated from four regions of the stomach, namely, the antrum, corpus, fundus, and incisura of individual patients. The possible correlation of Le antigen expression with the presence of *cagA* is still contradictory (8, 10). We also analyzed the possible association of Le antigens expression with *cagA*.

Eight Mexican patients with an average age of 58 years (range, 28 to 71 years; five male, three female) presenting at the Gastroenterology Unit of Hospital de Especialidades, CMNSXXI, Instituto Mexicano del Seguro Social, in Mexico City because of gastroduodenal symptoms were studied. Participants were informed about the nature of the study and asked to sign a consent form; the present study was approved by the Instituto Mexicano del Seguro Social Ethics Committee. Two biopsy samples from each site—the antrum, incisura, corpus, and fundus—were obtained; one biopsy of each site was homogenized and cultured for *H. pylori* on selective blood agar medium in a 10% CO₂ atmosphere, as previously described (6). A total of 226 individual colonies were studied (Table 1), a mean of 28 colonies per patient, and seven patients per region. From each colony, a 48-h culture in blood-agar was harvested in saline solution for DNA isolation and for Le antigen determination. The presence of Le antigens was determined by an enzyme-linked immunosorbent assay using commercial monoclonal antibodies to Le^x and Le^y (Signet Laboratories, Dedham, MA), as previously described (5). Results were expressed in optical density units (ODU), and values of >100 ODU were considered positive. Each clone was tested in

quadruplicate on 2 different days, and the mean of the ODU was used for analysis. Differences in frequencies among regions were analyzed by using the chi-square test. DNA was isolated by using guanidine-isothiocyanate-sarkosyl and then used to determine *cagA* by PCR (6) and to genotype isolates by RAPD [random(ly) amplified polymorphic DNA] as previously described (1).

In contrast to previous studies (6–8, 10), we mapped the expression of Le antigens by strains colonizing four regions of the stomach. The frequencies of expression of Le antigens among all of the 226 isolates were 38% for Le⁻, 28% for Le^y, 27% for Le^{xy}, and 7% for Le^x (Table 1). It is important to note that the frequency of colonies expressing Le^{x+y-} was significantly lower ($P = 0.02$) in the antrum than in the fundus. In addition, there was a trend for colonies Le^{x-y-} to be less common in the antrum than in the incisura and fundus, although this trend was not significant. Taken together, the frequency of colonies expressing Le^y was significantly higher in antrum (38 of 57) than in both incisura and fundus (27 of 56 in both) ($P = 0.048$) (Table 1). In addition, there was a trend for colonies expressing Le^{x+y+} to be more frequent in the antrum than in the fundus, although this trend was not significant ($P = 0.15$).

As in a previous study in Mexican patients (6), a considerable variability in the levels of expression of Le antigens (mean \pm the standard deviation) was found in all colonies from the four regions of the stomach (Fig. 1 and Table 2), even among isolates from the same patient. For example, the isolates from patient 256 were highly variable for Le^y, and the isolates from

TABLE 1. Frequency of Le antigen expressed by 226 isolates from four different regions of the stomachs of eight Mexican patients

Region	No. of colonies	No. (%) of isolates with Lewis antigen type:			
		Le ^{x+y-}	Le ^{x-y+}	Le ^{x+y+}	Le ^{x-y-}
Antrum	57	1 (2)	18 (32)	20 (35)	18 (31)
Incisura	56	5 (9)	14 (25)	13 (33)	24 (43)
Corpus	57	4 (7)	17 (30)	15 (26)	21 (37)
Fundus	56	6 (11)	14 (25)	13 (23)	23 (41)
Total	226	16 (7)	63 (28)	61 (27)	86 (38)

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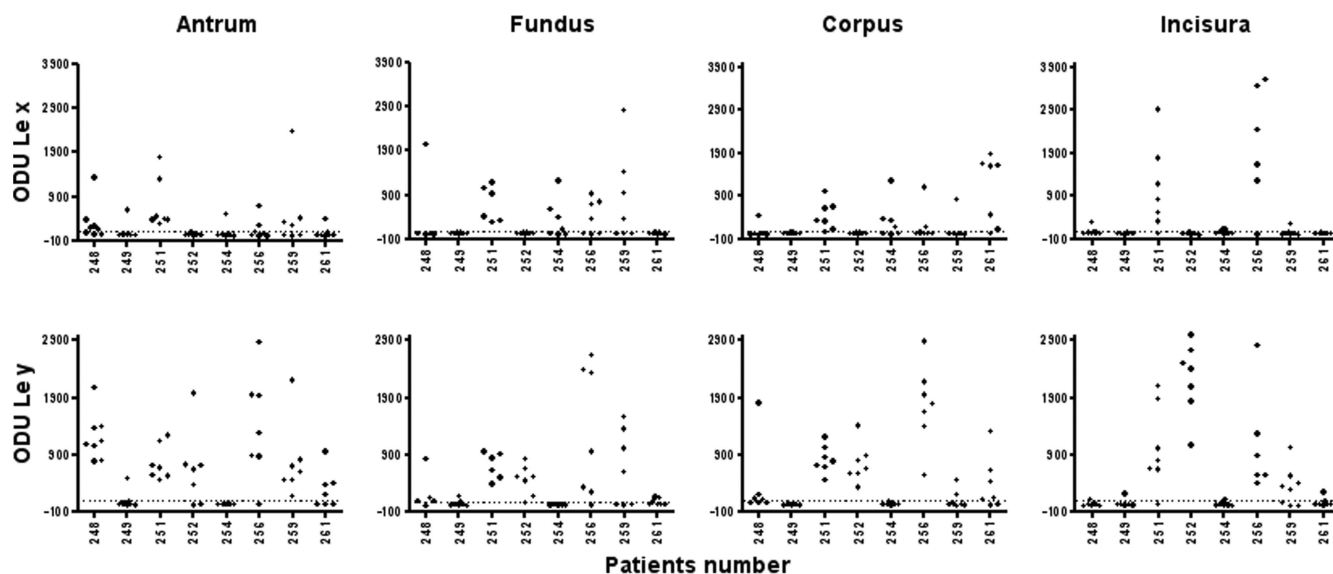


FIG. 1. Expression of Le^x and Le^y antigens by 226 *H. pylori* colonies isolated from the antrum, fundus, corpus, and incisura of the stomachs of eight Mexican patients.

patient 251 were highly variable for Le^x in the four stomach regions studied. Even so, in the antrum the level of expression of Le^y (972 ± 621) was significantly higher than the expression of Le^x (599 ± 578), whereas in the other three regions no significant difference was found between the expressions of Le^y and Le^x. The variability observed in the expression of Le antigen was not due to colonization with different strains, since in seven of the eight patients all of the isolated colonies showed the same RAPD pattern, and only in one case (patient 259) was a mixed infection documented, showing two different RAPD patterns. In accordance with these results, a previous study also reported a high diversity in the expression of Le antigens among multiple isolates from single patients even if they had the same RAPD pattern; the authors of that study suggested that variability in expression of Le antigens is not due to genetic diversity but to mechanisms regulating the expression and activity of fucosyltransferases (11). This variability would justify considering each colony as a phenotypically independent event for the statistical analyses.

The results presented above suggest that there is selective expression of Le antigens by *H. pylori* in the different regions of the stomach. The expression of Le^y seems to be predominant, or selected for in the antrum, whereas that of Le^{x-y-} and Le^x would be favored in the environment of the fundus. A limita-

tion of the present study is the number of patients analyzed, and a study with larger number of cases is needed to confirm these results. It has been suggested that the expression of surface antigens involved in interaction and binding to epithelial cells, such as Le antigens by *H. pylori*, is influenced by the environment present in the different regions of the gastroduodenal regions (9). Thus, a previous study found significant differences between strains colonizing the antrum and those colonizing the duodenum (9).

The expression of Le antigens has been associated with the presence of the *cagA*⁺ gene. Le^x expression has been reported as associated to *cagA*⁺ (10); however, and similar to other studies (4, 6, 8), we did not find such an association. In fact, even considering that Le^x was the less expressed of the Le antigens (16 of 226 isolates, Fig. 1), only 4 (25%) of the 16 Le^x colonies were *cagA*⁺. These four colonies were isolated from three different patients. We found a significant decreasing tendency of association with *cagA* and Le antigens as follows: Le^x < Le^y ($P = 0.02$) < Le^{xy} ($P = 0.005$) < Le⁻ ($P < 0.001$). Thus, in contrast to previous studies, *cagA* was significantly more associated with strains not expressing Le antigens.

In contrast to what we found for Le expression, the presence of *cagA*⁺ strains was not significantly different among the four regions of the stomach studied, which is consistent with previous results of an in situ study of *cagA*⁺ strains (2).

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TABLE 2. Comparison of levels of expression of Lewis antigens in four different regions of the stomach by single-colony isolates from eight Mexican patients

Region	Expression of Le antigen (mean ODU \pm SD)		P
	Le ^x	Le ^y	
Antrum	599 \pm 578	972 \pm 621	0.025
Incisura	1,301 \pm 1,13	1,156 \pm 926	0.65
Corpus	752 \pm 575	901 \pm 684	0.42
Fundus	885 \pm 665	820 \pm 694	0.75

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