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Phenotypic Variation of Helicobacter pylori Isolates from Geographically Distinct Regions Detected by Lectin Typing

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A total of 309 Helicobacter pylori isolates from 18 different countries were analyzed with a previously developed lectin typing system. The system was developed by using a proteolytic pretreatment to enhance the carbohydrate fraction of the sample. Four lectins from Ulex europaeus, Lotus tetragonolobus, Erythrina cristagalli, and Triticum vulgare were used to type the strains. The lectins were chosen for their specificities for sugars commonly encountered in the lipopolysaccharide of H. pylori. The isolates were received from their parent institutions as pellets of biomass and were typed at one of three centers (in Ireland, Sweden, and Estonia). All 16 possible lectin reaction patterns were observed in the study, with the isolates having the predominant pattern exhibiting reactions with all the lectins in the panel. For European patients suffering from gastritis, an association was noted between lectin reaction pattern MH4 and atrophic chronic gastritis; isolates with lectin reaction pattern MH1 and MH10, which had high lectin reactivities, were associated with ulcer disease (P = 0.0006 and P = 0.0025, respectively), and all four isolates from adenocarcinoma patients were characterized as possessing reaction pattern MH16. In contrast, isolates with lectin reaction patterns MH1 and MH10, which had high lectin reactivities, were associated with ulcer disease (P = 0.046 and P = 0.0022, respectively).

Infection with Helicobacter pylori is associated with a number of pathologies, including chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (9). The reason for these differences in clinical outcomes associated with H. pylori infection and the reason why some infected individuals remain asymptomatic are not known. It has been suggested that host or environmental factors may play a role in these differences or that certain more virulent strains of H. pylori exist (6, 29).

Numerous typing systems have been used to differentiate and group strains of H. pylori. The majority of these procedures have involved genotypic typing methods, which have noted differences between strains of H. pylori isolated from distinct host populations (4, 14, 18, 25, 31). The results of these studies strongly suggest that host-adapted strains exist. In addition, genotypic characteristics such as the presence of the cag pathogenicity island and the presence of vacA type s1a and expression of the cytotoxin-associated gene A product (CagA) or vacuolating toxin (VacA) have been shown to be markers for specific pathologies in certain populations (3, 24, 29, 32). However, such associations have not been found for all populations (12, 17).

A number of phenotypic typing systems for H. pylori, including serotyping, are based upon variation in the O side chain of lipopolysaccharide (LPS) of the bacterium, especially the expression of Lewis (Le) blood group determinants (19, 26). Several serological studies have suggested that Le antigens may act as markers for strains associated with specific pathologies, in particular, ulcer disease (8, 17, 32; N. Brouet, XIIIth Int. Workshop Gastrointestinal Pathology and Helicobacter pylori, Gut 47[Suppl. I]:A27–A28, 2000). Previously, a novel phenotypic typing system based on lectin typing which can be successfully applied to H. pylori was developed (10). This system has advantages over serotyping, particularly with anti-Le monoclonal antibodies. First, since antibodies for a conserved set of structures are used, such serotyping may exhibit a low level of discriminatory ability; second, a large number (15%) of untypeable strains occur (26); third, technical expertise is required for the performance of the tests (19, 26); and fourth, inconsistencies between test systems that use anti-Le antibodies in different formats have been demonstrated (1, 11).
Moreover, the lectin typing system does not require the raising of antibodies in animals, as is required for serotyping, and is ethically sound, less time-consuming, and economically advantageous (10). Lectins have been proposed for use as diagnostic and typing tools in a number of studies for other bacteria (5, 13, 23, 34). The present system was developed to detect variations in carbohydrate expression by \textit{H. pylori} LPS. Moreover, the use of lectin-blotting analyses in a previous study (10) established that lectins in the typing system bind predominantly with carbohydrates in the accessible O-side-chain region of LPS but reactions with carbohydrates in the LPS core were not excluded. The system has proved reproducible, sensitive, and easy to use when applied to a group of strains isolated from a geographically distinct population in Ireland (10). The present study reports on the application of this typing system to a large number of isolates from different geographical locations worldwide. The aim of our study was to examine whether the host population or associated gastroduodenal pathology was reflected in phenotypic differences between strains of \textit{H. pylori} as detected by the lectin typing system.

MATERIALS AND METHODS

Origins of bacterial samples. \textit{H. pylori} was isolated from antral biopsy material from 309 patients presenting at open-access endoscopy units and undergoing upper gastrointestinal endoscopy. The patients were from 18 different countries, and each patient presented in his or her home country with dyspeptic symptoms. The patients exhibited a variety of endoscopy-diagnosed disorders, including gastritis, peptic ulcer disease, atrophic chronic gastritis (ACG) with or without intestinal metaplasia (IM) (ACG ± IM), and gastric adenocarcinoma. Patients presenting with gastritis were classified by using the Sydney system (27). For inclusion in the European cohort (Eurohepgast Study Group) of ACG ± IM patients, patients had to present themselves in one of the participating centers, to be between 18 and 75 years of age, and to have had an endoscopy in which a biopsy diagnosis of chronic gastritis was made. In addition, a small proportion (3%) of the bacterial isolates were obtained from asymptomatic patients. The country or population of origin and the number of the total of 309 \textit{H. pylori} isolates from each country were as follows: Belgium, \( n = 1 \); Bulgaria, \( n = 17 \); China, \( n = 12 \); Czech Republic, \( n = 8 \); Estonia, \( n = 28 \); Finland, \( n = 4 \); France, \( n = 18 \); Germany, \( n = 9 \); Greece, \( n = 35 \); Hungary, \( n = 1 \); Ireland, \( n = 13 \); Italy, \( n = 4 \); Japan, \( n = 20 \); the Polynesian population of New Zealand, \( n = 10 \); Poland, \( n = 13 \); Portugal, \( n = 16 \); Spain, \( n = 19 \); and Sweden, \( n = 81 \).

\textbf{Bacteria and culture conditions.} The bacteria were routinely grown on 5% blood agar under microaerobic conditions with 10% CO\(_2\) and 5% O\(_2\), and the debris. The resultant pellet was resuspended in PBS to an

\textbf{Results}

\textbf{RESULTS}

Reproducibility. All strains were lectin typed at the Depart-ment of Microbiology, National University of Ireland, Galway, which was the centralized facility. In addition, two independent subsets of these strains, 116 (38%) and 19 (6%) strains, respect-ively, were retested at two other test centers in Sweden (Department of Medical Microbiology, Dermatology and Infection, Lund University) and Estonia (Department of Medical Microbiology, University of Tartu) and yielded lectin reaction patterns identical to those observed at the centralized facility. Lectin typing of each extract was repeated in triplicate, and, in addition, strains that had been regrown from stocks were lectin typed again and gave identical results. The method proved to be robust and was readily applied to all the \textit{H. pylori} isolates and in all the test laboratories. Furthermore, at the centralized facility, pellets of biomass that had been received and subcultures of frozen stocks gave identical results (data not shown).

\textbf{Lectin reaction patterns.} By using a panel of four lectins, a total of 16 distinct lectin reaction patterns are possible, and these were given arbitrary designations (Table 1) similar to those used in a previous study (10). Examples of all 16 possible reaction patterns were observed. The most common lectin reaction pattern detected was MH1, but other common patterns were MH5, MH10, MH13, and MH16 (Table 2). Isolates with pattern MH1 reacted with all the \textit{H. pylori} lectins and in all the test laboratories. Furthermore, at the centralized facility, pellets of biomass that had been received and subcultures of frozen stocks gave identical results (data not shown).

\textbf{Lectin reaction pattern and geographical variation.} For meaningful analysis, strains were grouped into regions on the basis of their country of isolation. Five regions were delineated: Northern Europe comprised Estonia, Finland, and Swed-en; Central Europe comprised Belgium, France, Germany, and Ireland; Eastern Europe comprised Bulgaria, the Czech
Republic, Hungary, and Poland; Southern Europe comprised Greece, Italy, Portugal, and Spain; and the Pacific region (Asia and Oceania) comprised China, Japan, and the Polynesian population of New Zealand.

The occurrence of the 16 possible lectin reaction patterns in the different regions is shown in Table 2. Isolates from the Pacific region and Eastern Europe had a notably smaller variety of reaction patterns than those from Central, Southern, or Northern Europe. In particular, pattern MH1 was the most common pattern among Pacific region isolates, but it was also the most common among isolates from Central, Southern, and Eastern Europe. In contrast to the other regions, pattern MH10 occurred as frequently as pattern MH1 among Northern European isolates. Pattern MH16, isolates of which are nonreactive with any lectins in the panel, also was common among Northern European isolates, but none of the Eastern European isolates had pattern MH16. Pattern MH9 was specific for the Eastern European isolates and occurred only in isolates from Poland and Bulgaria. Examination of the distribution of the lectin reaction patterns among the individual countries of the Northern European region demonstrated that whereas pattern MH10 was the most common among Swedish isolates, pattern MH5 was the most common among Estonian isolates and pattern MH16 was present only in Swedish isolates. In contrast, the Finnish isolates examined had a distribution of reaction patterns similar to those of isolates from other European countries, with pattern MH1 being the most common pattern.

**Lectin reaction pattern and associated pathology.** The majority of the European isolates (n = 267) were from patients with either chronic gastritis or ACG ± IM alone. Quantitative histological data for isolates from 90 of these patients were available, and these were subjected to further analysis. Data were provided for both the antral and the fundic regions of the patients’ stomachs and included parameters such as degree of inflammation, occurrence of ACG ± IM, surface epithelial damage, *H. pylori* colonization density, and production of lymphoid follicles. Further quantitative histological information was available for the duodenal region, including parameters similar to those described above: villous atrophy, chronic inflammation, acute inflammation, occurrence of antral IM, *H. pylori* colonization density, and lymphoid follicle production. No lectin reaction pattern statistically correlated with any of the histological findings listed above for the antral or fundic regions or the duodenal regions of *H. pylori*-infected patients. Analysis of European strains was limited to comparisons between the distributions of the lectin reaction patterns of isolates from chronic gastritis patients and patients with ACG ± IM (Table 3). Although only five isolates were typed as MH4,
TABLE 4. Lectin reaction patterns of 76 *H. pylori* isolates from the Swedish population and the host’s associated pathology

<table>
<thead>
<tr>
<th>Lectin reaction pattern</th>
<th>No. (%) of isolates from patients with the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonulcer disease</td>
</tr>
<tr>
<td>MH1</td>
<td>4 (8.9)</td>
</tr>
<tr>
<td>MH2</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>MH3</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>MH4</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>MH5</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>MH6</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>MH7</td>
<td>2 (4.4)</td>
</tr>
<tr>
<td>MH8</td>
<td>4 (8.9)</td>
</tr>
<tr>
<td>MH9</td>
<td>3 (6.7)</td>
</tr>
<tr>
<td>MH10</td>
<td>12* (26.7)</td>
</tr>
<tr>
<td>MH11</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>MH12</td>
<td>16* (35.6)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (100)</td>
</tr>
</tbody>
</table>

* The result is considered significant (*P* < 0.05) by Fisher’s exact test.

A relationship was observed between isolates with lectin reaction pattern MH4 and ACG ± 1M, in which isolates with lectin reaction pattern MH4 were isolated from patients with ACG ± 1M, whereas isolates with this pattern were not isolated from patients with chronic gastritis (*P* = 0.0006) (Table 3). In addition, a relatively large proportion (92%) of isolates with pattern MH5 were associated with chronic gastritis, but this was not statistically significant (*P* = 0.28) compared with the other lectin reaction patterns detected in association with chronic gastritis and ACG ± 1M.

The Swedish isolates examined in the present study were isolated from patients with dyspeptic symptoms presenting for upper gastrointestinal endoscopy at an open-access endoscopy unit. As shown in Table 4, strains were selected on the basis of their availability from patients with gastritis (nonulcer disease), ulcerative disease, and gastric adenocarcinoma. Within this group of Swedish patients, isolates with lectin reaction pattern MH1 occurred significantly more often (*P* = 0.047) in those with ulcerative disease than in those with nonulcerative disease. Moreover, a stronger statistical relationship (*P* = 0.002) existed between pattern MH10 and ulcerative disease (Table 4). In contrast, lectin reaction patterns MH13 and MH16 occurred significantly more often (*P* = 0.002 and *P* = 0.0002, respectively) in isolates from Swedish patients with nonulcerative diseases than in isolates from Swedish patients with ulcerative diseases. Moreover, all four isolates from patients with adenocarcinoma, not defined as either an ulcer or a nonulcer disease, exhibited pattern MH16, isolates of which are unreactive with any of the lectins in the panel.

**DISCUSSION**

It has been suggested that the expression of carbohydrates in the O-polysaccharide side chain of *H. pylori* LPS, especially the mimicry of human Le antigens, plays a number of potential roles in the pathogenesis of the bacterium (22). They may act in the evasion of host defenses, and subsequently, due to the chronic nature of the infection, their expression may play a role in autoimmune aspects of pathology (22). Also, it has been suggested that these antigens act in adhesion of the bacterium to the gastric mucosa (2, 7, 21). Furthermore, it has been reported that the expression of these fucosylated blood group antigens by *H. pylori* is related to the host Le phenotype (33), suggesting that carbohydrate expression by the bacteria adapts to the host environment. However, conflicting results have also been reported (8, 28).

The results of the present study indicate that considerable microdiversity exists in carbohydrate expression in the O side chain and LPS of *H. pylori*. In previous studies with antibodies, Mills et al. (19) developed a serotyping system using passive hemagglutination but could differentiate only six serotypes. Also, the extraction procedure used to produce the antigenic material and the technique used to titrate the antisera were time-consuming and labor-intensive, and the investigators concluded that they were unsuitable for use in the clinical laboratory. Nevertheless, in related immunoblotting studies, Mills et al. (19) demonstrated variations in the reactions of LPSs, particularly the O side chains, from isolates of various serotypes with their typing antisera. Furthermore, Simoons-Smit et al. (26) used four anti-Leα and anti-Leα monoclonal antibodies with different fine specificities in an enzyme-linked immunosorbent assay to differentiate strains into 12 different serotypes. However, a large proportion of isolates (15%) remained untypeable with anti-Le antibodies. In contrast to monoclonal antibodies that have been used to detect Le antigens (8, 11, 26, 32), lectins do not require complete epitopes for binding and may therefore detect more subtle differences in carbohydrate structure (5, 34). Previously, we have suggested that the Anguilla anguilla lectin, which has a specificity for fucose monosaccharide, may be used to subtype certain lectin reaction patterns (10). However, in the present study the ubiquitous reactivity of this lectin with all isolates examined (data not shown) meant that it was of little use for differentiation of strains. By the use of four lectins, all 16 possible lectin reaction patterns were observed in the course of the study, illustrating the presence of greater variation in carbohydrate expression by strains than that detectable with anti-Le monoclonal antibodies (8, 11, 26, 32).

The most common lectin reaction patterns observed were MH1, MH5, MH10, and MH13. All of these patterns exhibit reaction with at least LotusA and WGA, which have specificities for fucose and N-acetylgalactosamine, respectively. However, the ability to differentiate between these isolates by the use of other lectins confirms the high degree of microdiversity in carbohydrate expression occurring in the LPS of *H. pylori*.

Also, the lectin reaction patterns of *H. pylori* isolates were analyzed in the context of their geographical region of isolation, and strain diversity was reflected in a number of region-specific variations in *H. pylori* lectin reaction patterns. No two regions or countries had the same frequency of distribution of patterns. Nevertheless, a region-specific reaction pattern was observed for isolates from Eastern Europe; pattern MH9 was observed only in isolates from Bulgaria and Poland. When data for the patients infected with these three isolates were more closely scrutinized, it was apparent that apart from their geographical origin, all three patients presented with common
pathologies and blood groups (acute gastritis, blood group A, and Rh factor positive). Although the number of isolates involved was small, it indicates the potential for use of lectin typing for the identification of geography-specific reaction patterns and may be indicative of the adaptation of strains to the host (4, 33). Greater variations in reaction patterns were associated with Northern, Central, and Southern European isolates. These greater variations in phenotypes may reflect the greater heterogeneity of these host populations. Conversely, isolates from populations with similar ethnic backgrounds, such as populations from China and Japan, had similar, but not identical, frequencies of lectin reaction patterns. Campbell et al. (4) showed that H. pylori strains isolated from the Polynesian and European descendant populations of New Zealand were genetically distinct. The MH1 pattern was the most common reaction pattern among isolates from the Pacific region, including Polynesian isolates, examined in the present study, as was the case for isolates from certain European populations; in contrast, the isolates from the Pacific region had relatively few lectin reaction patterns. These phenotypic variations of H. pylori which coincide with differences in human populations would argue for the presence of host-adapted strains (6). The presence of regional differences between H. pylori isolates observed in the present study is in agreement with the findings of a number of studies that have demonstrated genotypic differences between Asian and European isolates of H. pylori (12, 18, 31, 36). Moreover, Monteiro et al. (20) have shown in structural studies of LPS that although Le antigens are commonly expressed by strains from both the European and the Asian populations, there is a preponderance of Le<sub>a</sub> and Le<sub>b</sub> over Le<sub>c</sub> and Le<sub>ε</sub> isoforms among Asian isolates. This agrees with data from the present study, which show a predominance of lectin reaction pattern MH1 among isolates from the Pacific region (Asia and Oceania), indicating the common occurrence of Le-related carbohydrate structures in this geographical region. Furthermore, similar to LPS structural studies, serological analyses with anti-Le antibodies have shown geographical differences in the expression of Le antigens by H. pylori strains, in particular between Asian and European isolates in the expression of Le<sub>a</sub> and Le<sub>b</sub> antigens (1, 8, 17, 26, 35, 36), although some isolates remain untypeable with anti-Le antibodies (8, 11, 26, 32). Importantly, the present lectin typing system has detected greater diversity among isolates than has been detectable with anti-Le antibodies (26) and has highlighted geographical differences in carbohydrate expression by H. pylori isolates due to its capacity for broader recognition of variations in carbohydrate structures (10).

In the present study, detailed histopathological data were available only for isolates from European (Eurohepygast Study Group) patients suffering from chronic gastritis and ACG ± IM. Therefore, this result is in agreement with data from other investigations (8, 17, 32) which have all associated Le antigen expression with an aggressive pathology, including ulceration. In addition, a Japanese study has shown that the expression of LPS by H. pylori with a low level of antigenicity may be associated with the development of gastric cancer (35) and that strains unreactive with anti-Le antibodies exist in this population (1). Similarly, this low level of antigenicity may be manifest in a low level of reactivity with lectins, as evidenced by the association of isolates with pattern MH16 (which do not react with any lectins) with gastric adenocarcinoma and the association of isolates with pattern MH4 (which react only with ECA lectin) with ACG ± IM. This association may be indicative of a link between isolate lectin reaction pattern and disease. In addition, since conditions in the stomach change upon the development of ACG ± IM and other bacteria can proliferate in this environment, the association may instead indicate that H. pylori strains with pattern MH4 could be better adapted to these new conditions.

Collectively, our data show that lectin typing is an effective method for epidemiological studies. It was successfully applied to a large and diverse group of H. pylori isolates at multiple centers, and the study proved its ease of application. Moreover, the system as applied was capable of demonstrating phenotypic differences between populations of H. pylori strains.

**APPENDIX**

Members of the Eurohepygast Study Group include the following: F. Mégraud (Bordeaux, France), C. O’Morain (Dublin, Ireland), P. Sipponen (Espoo, Finland), A. Price (London, United Kingdom), P. Malfertheiner (Magdeburg, Germany), N. Munoz (Lyon, France), F. Dabis (Bordeaux, France), N. Figura (Siena, Italy), M. Plebani (Padua, Italy), C. Sakarowitch (Bordeaux, France), F. Nachit (Bordeaux, France), I. M. Arenas (San Sebastian, Spain), A. Blasi (Catania, Italy), J. F. Bretagne (Rennes, France), S. Chaussade (Paris, France), J. C. Delchier (Créteil, France), J. D. De Korwin (Nancy, France), D. Duda (Wroclaw, Poland), J. Dziemiszewski (Warsaw, Poland), B. Fixa (Hradec Kralové, Czech Republic), X. Gisbert (Madrid, Spain), A. Goldis (Timisoara, Romania), J. Guerre,
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