Helicobacter pylori isolates from Greek children express type 2 and type 1 Lewis and α 1,6-glucan antigens in conjunction with a functional type IV secretion system

Eleonora Altman,¹ Vandana Chandan,¹ Blair A. Harrison,¹
Effrosini G. Panayotopoulou,² Eleftheria Roma-Giannikou,³
Jianjun Li¹ and Dionyssios N. Sgouras²

Correspondence

Eleonora Altman

Eleonora.altman@nrc-cnrc.gc.ca

Dionyssios N. Sgouras

sgouras@pasteur.gr

¹Institute for Biological Sciences, National Research Council Canada, Ottawa, ON K1A 0R6, Canada

²Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens 11521, Greece

³First Department of Pediatrics, Athens University School of Medicine, Athens, Greece

Helicobacter pylori infection is often acquired in childhood and can persist for life. Previous studies in adult patients have shown that *H. pylori* isolates from North American and European hosts express predominantly type 2 Lewis x (Le^x) and Le^y epitopes, while Asian strains have the capacity to express type 1 Le^a and Le^b structures. In order to understand the influence of environmental and host factors on the expression of Le antigens, we analysed 50 Greek *H. pylori* isolates from symptomatic children. Both CagA-positive and -negative strains were evaluated. The expression of Le antigens was determined by whole-cell indirect ELISA (WCE), and LPS profiles were assessed by gel electrophoresis and immunoblotting. Occurrence of Le^x and/or Le^y antigens was confirmed in 35 of the isolates (70 %) while 15 of the isolates were non-typable. It was found that 11 of the paediatric isolates had the propensity to express type 1 Le^b blood-group antigen (22 %), a feature relatively uncommon in *H. pylori* isolates from adults. One strain expressed both Le^b and Le^a antigens. The majority of the isolates (49/50, 98 %) expressed α 1,6-glucan, an antigenic non-Le determinant present in the outer core region of *H. pylori* LPS. All Le^x- and Le^y-expressing strains also carried a functional *cag* pathogenicity island-encoding a type IV secretion system, capable of translocating CagA protein, as well as the *vacAs1* allele, suggesting that Le^x and Le^y epitopes may aid the persistence of more aggressive strains. No association between bacterial virulence characteristics and the histopathological observations was evident.

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INTRODUCTION

Helicobacter pylori has been associated with chronic infection of human gastric mucosa and increased risk of gastric cancer and peptic ulcers in adults (Dunn *et al.*, 1997). The vast majority of individuals acquire *H. pylori* in childhood. The infection often remains asymptomatic and, if left untreated, can persist into adulthood, leading to more serious pathologies. Lipopolysaccharide (LPS) is a major cell-surface component of *H. pylori*. Structural studies carried out on a number of *H. pylori* isolates (Monteiro, 2001) have resulted in a structural model of LPS, consisting of an O-chain and a core oligosaccharide

that is attached to a lipid A moiety. The O-chain of the LPS in a number of *H. pylori* strains from North American and European populations expresses type 2 Le^x and Le^y blood-group epitopes (Monteiro, 2001) that mimic structures present on human gastric and tumour cell surfaces and could be implicated in adverse autoimmune reactions with the host (Appelmelk *et al.*, 1996). Recently, it has been suggested that *H. pylori* can change its Le phenotype to the type 1 pathway *in vivo* in order to adapt to specific conditions in the host (Pohl *et al.*, 2009). Furthermore, it has been suggested that depending on the human host population, Le^x/Le^y expression by *H. pylori* may aid the persistence of more aggressive strains (Moran, 2008).

In the present study, we evaluated LPS expression in 50 *H. pylori* strains from the Greek paediatric population, since children constitute a natural milieu less influenced by environmental and host-associated factors (Oleastro *et al.*,

Abbreviations: CE-MS, capillary electrophoresis-mass spectrometry; CI, confidence interval; HRP, horseradish peroxidase; LacNAc, *N*-acetyllactosamine; OD, odds ratio; PUD, peptic ulcer disease; T4SS, type IV secretion system; WCE, whole-cell indirect ELISA.

2010). The isolates were screened by whole-cell indirect ELISA (WCE) using commercially available anti-Le monoclonal antibodies (mAbs) and anti- α 1,6-glucan-specific mAbs developed in our laboratory (Harrison *et al.*, 2011). Selected strains were further characterized in terms of carbohydrate content by using SDS-PAGE electrophoresis, immunoblotting and chemical and mass spectrometric analyses. Other virulent characteristics, such as vacA isoforms, presence and functionality of type IV secretion systems (T4SS) and the number and type of functional EPIYA-phosphorylation motifs on translocated CagA proteins, were correlated with the LPS content.

METHODS

Patients. The study included symptomatic *H. pylori*-infected children, 2–16 years old, who underwent upper endoscopy at the gastroenterology clinic of the First Department of Paediatrics of Athens University, Aghia Sophia Children's Hospital, as detailed before (Sgouras *et al.*, 2009). The study was approved by the Hospital Scientific Committee and the Ethical Committee.

Culture. Antral mucosa biopsy specimens were processed for *H. pylori* isolation as described previously (Sgouras *et al.*, 2009). Individual colonies were further propagated, and frozen stocks were prepared using BHI broth containing 20% (v/v) glycerol. They were stored at -80°C until further analysis.

Whole-cell indirect ELISA (WCE). *H. pylori* strains were cultivated on plates as described previously (Logan *et al.*, 2000). Cells were harvested and washed with 5 ml 10 mM PBS (pH 7.4) per plate. Following centrifugation (5000 g, 5 min, 4°C), pellets were suspended in 25 ml PBS to give a final concentration of 10^8 cells ml^{-1} . WCE was performed as previously described (Altman *et al.*, 2008). Briefly, the wells of a microtitre plate were coated with 100 μl bacterial suspension (10^8 cells ml^{-1}) overnight at 4°C . The wells were then fixed with methanol and blocked with 200 μl Milk Diluent/Blocking solution (MDB) (KPL) for 2 h at 37°C . Subsequently, the wells were incubated for 2 h at 37°C with either 100 μl anti-Le^a, -Le^b, -Le^x or -Le^y mAb solution (Signet Laboratories) diluted 1:200 in MDB, 100 μl 1C4F9 ascites (anti- α 1,6-glucan mAb) (Harrison *et al.*, 2011) diluted 1:500 in MDB or rabbit anti-CagA antibody (Austral Biologicals) diluted 1:100 in MDB. This was followed by incubation with horseradish peroxidase (HRP)-conjugated anti-mouse IgG + IgM (Caltag) diluted 1:1000 in MDB for 1 h at room temperature. A corresponding secondary antibody, either HRP-anti-mouse IgG + IgM or HRP-anti-rabbit IgG (Cedarlane Laboratories) diluted 1:1000 in MDB, was added and the plates were incubated for 1 h at room temperature. The substrate TMB was added as described previously for indirect ELISA (Altman *et al.*, 2005). OD₄₅₀ values <0.2 were classified as negative and OD₄₅₀ values ≥ 0.2 were classified as positive reactions. To ensure consistency between plates, *H. pylori* strain 26695 cells were used as a positive control for Le^x, Le^y, α 1,6-glucan and CagA. Assay results did not vary by more than 10%.

Electrophoresis and immunoblotting. SDS-PAGE was performed with a mini-slab apparatus (Bio-Rad) according to the method of Laemmli (1970). LPS samples were prepared from whole cells according to a previously described method (Logan & Trust, 1984). Equivalent amounts of sample were loaded into each lane and stained according to Tsai & Frasch (1982). For detection of Le^b in bacterial whole-cell samples, nitrocellulose membranes were blocked with 1% skimmed milk solution in 10 mM PBS overnight at 4°C . Subsequently, membranes were incubated with anti-Le^b mAb solution

diluted 1:100 in 10 mM PBS, overnight at 4°C . The corresponding secondary antibody HRP-anti-mouse IgG + IgM diluted 1:3000 in 10 mM PBS was added, and the nitrocellulose membranes were incubated for 1 h at room temperature. The membranes were washed three times with 10 mM PBS between the incubation steps and developed as described previously (Altman *et al.*, 2008) using SuperSignal West Pico Chemiluminescent substrate (Pierce), following the manufacturer's instructions.

Sugar analysis. Bacterial cells were hydrolysed using 2 M trifluoroacetic acid at 100°C for 16 h followed by reduction with sodium borohydride and subsequent acetylation as previously described (Altman *et al.*, 2003).

CE-MS. Bacterial cells were digested with RNase and DNase (final concentration $10\text{ }\mu\text{g ml}^{-1}$ in 0.02 M ammonium acetate, pH 7.5) for 2 h at 37°C to release LPS, and the cell pellets were delipidated with 0.1 M sodium acetate buffer (pH 4.5) (Altman *et al.*, 2003). Samples were desalted using a centrifugal filter device (Micron-3K, molecular mass cut-off 3000; Pall Corporation). The recovered solution was analysed directly by a Crystal model 310 CE instrument coupled to an API 3000 mass spectrometer (Li *et al.*, 2005).

Determination of *H. pylori* virulence characteristics. EPIYA-C motifs in CagA and the VacA vacuolating isoforms were assessed as previously described (Panayotopoulou *et al.*, 2010; Sgouras *et al.*, 2009). Functionality of the T4SS was assessed by testing for the presence of *cagA* and *cagE* genes, detected by PCR, and the phosphorylated CagA protein and secreted IL-8 in the supernatant of *H. pylori*-infected AGS cells (Panayotopoulou *et al.*, 2010; Sgouras *et al.*, 2009).

Statistical analysis. Contingency analysis was performed, and levels of significance were verified by univariate logistic regression analysis; selection of variables was based on a likelihood ratio test, using a significance level of 0.05 for inclusion or elimination.

RESULTS

LPS immunotyping of *H. pylori* isolates

Isolates were initially screened by WCE with anti-Le^x, -Le^y, -Le^a, -Le^b and - α 1,6-glucan mAbs. The expression of Le^x and/or Le^y antigens was detected in 35 strains, whilst 15 strains were non-typable (Table 1). Exclusive Le^b expression was identified in three of these non-typable isolates, whilst the presence of Le^b antigen was detected in 11 isolates in total, the further eight being either co-expressed with Le^x and Le^y (four isolates) or together with Le^y (four isolates). Co-expression of Le^x, Le^y, Le^b and Le^a antigens was confirmed in one strain (strain 103). The expression of type 2 and/or type 1 Le antigen was confirmed in 15 CagA-negative isolates (Table 1).

The presence of α 1,6-glucan was confirmed in 49 of the 50 strains (98%); the expression of this antigen in one strain (strain 68) was found to be slightly below the detection limit. All non-typable *H. pylori* strains expressed α 1,6-glucan, regardless of their CagA status (Table 1).

Structural characterization of non-typable strains

Non-typable *H. pylori* strains were further characterized by SDS-PAGE, sugar analysis and capillary electrophoresis-mass

Table 1. Analysis of paediatric *H. pylori* isolates from Greece with reference to expression of Lewis antigens, α 1,6-glucan and *cagA* status, number of EPIYA-C motifs in CagA, functionality of T4SS and vacuolation potential of VacA

Strain*	Le ^x	Le ^y	Le ^a	Le ^b	α 1,6-glucan	CagA Ag	No. EPIYA-C motifs†	T4SS‡	Vacuolation§
32	—	—	—	—	1.502	—	0	—	—
34	—	—	—	—	0.948	—	0	—	—
35	1.318	1.235	—	—	0.338	1.035	1	+	+
40	—	—	—	—	1.501	—	0	—	—
42	1.601	1.009	—	—	0.463	1.244	1	+	—
59	0.363	1.465	—	1.379	1.792	—	2	—	+
63	1.653	1.350	—	—	1.615	1.156	2	+	+
68	1.045	1.181	—	—	0.194	1.262	2	+	—
71	—	—	—	—	1.401	—	2	—	—
72	—	—	—	—	1.056	0.766	1	+	—
73	1.617	1.034	—	—	1.486	—	0	—	—
77-2	—	—	—	—	1.308	0.718	0	+	+
77-5	—	—	—	—	1.365	1.172	1	+	+
79	—	0.386	—	0.250	1.578	—	0	—	—
87	0.501	1.133	—	—	1.709	1.494	2	+	+
92	—	1.113	—	—	0.336	1.451	1	+	+
93	0.623	1.414	—	—	0.597	1.462	1	+	—
94	1.580	1.113	—	—	0.683	1.364	1	+	+
97	0.507	0.956	—	—	1.223	0.399	1	+	+
98	—	0.641	—	—	0.883	1.471	1	+	+
99	—	—	—	0.716	0.260	—	2	—	+
100	1.433	1.058	—	—	1.463	1.399	2	+	+
101	0.752	1.451	—	—	1.570	1.508	2	+	+
102	—	—	—	0.752	0.297	—	0	—	—
103	1.022	0.641	0.312	0.765	0.913	—	0	—	—
104	—	0.366	—	—	1.381	—	0	—	—
105	0.869	1.171	—	—	1.677	—	0	—	—
107	—	0.785	—	—	1.609	—	0	—	—
108	1.014	1.160	—	1.032	0.726	—	0	—	—
109	—	1.342	—	1.563	1.684	—	0	—	+
110	—	—	—	—	0.244	—	0	—	—
117	1.029	1.446	—	—	0.311	1.387	2	+	+
140676	1.546	1.313	—	—	0.240	1.197	1	+	+
144180	1.684	1.235	—	—	1.619	0.488	1	+	+
146195-8	—	1.035	—	—	0.827	1.384	1	+	+
146195-13	—	—	—	—	1.688	0.866	2	+	+
147181	—	—	—	—	1.063	—	0	—	—
147403	1.701	1.539	—	0.370	0.463	1.133	2	+	+
147570	—	—	—	0.406	0.449	1.146	1	+	+
147631	—	0.601	—	—	0.395	1.146	2	+	—
148455	—	—	—	—	0.418	1.185	1	+	+
148715	1.769	1.545	—	—	0.441	—	0	—	—
149489	—	1.457	—	0.824	1.737	—	0	—	—
150453	1.633	1.538	—	—	0.489	0.930	1	+	+
150934	1.729	1.611	—	—	1.814	1.074	2	+	+
152047	1.040	1.435	—	—	1.602	—	2	—	+
153085	—	0.556	—	1.142	0.386	1.063	1	+	—
160708	—	—	—	—	1.810	—	0	—	—
160729	—	1.544	—	—	1.804	—	0	—	—
167005	1.597	1.378	—	—	0.508	1.106	2	+	+

*Clinical isolates belonging to the Hellenic Pasteur Institute Biobank (HPI reference number) as described before (Sgouras *et al.*, 2009).†Number of EPIYA-C motifs [RS(V/A)SPEPIYATIDDLG] in CagA, assessed as described before (Sgouras *et al.*, 2009).‡Functional status of type IV secretion system in *H. pylori* strains, assessed as described previously (Panayotopoulou *et al.*, 2010; Sgouras *et al.*, 2009).§Vacuolation potential of VacA was assessed as described previously (Panayotopoulou *et al.*, 2010).

spectrometry (CE-MS) performed directly on bacterial cells. Silver staining revealed significant variations in the O-chain length of these strains. Strains 32, 40, 71, 146195-13, 147181, 147570 and 160708 appeared to contain an extended O-chain component compared with the staining pattern of *H. pylori* strain 26695, although they tested negative for the presence of Le^y and Le^x antigens by WCE. Sugar analysis suggested that these isolates contained reduced amounts of L-fucose compared with the cells of *H. pylori* strain 26695, which are known to carry Le^x and Le^y antigens (Table 2). Silver staining patterns of strains 72, 77-2 and 77-5 appeared to be almost rough and showed only very faint high-molecular-mass bands (Fig. 1). It is of note that strains 40, 146195-13 and 147181 contained increased amounts of DD-heptose compared with strain 26695, suggesting the presence of DD-heptan in their LPS (Table 2) (Hiratsuka *et al.*, 2005). *H. pylori* strains 32, 146195-13 and 160708 contained the highest amount of D-glucose compared to 26695 cells, suggesting the presence of a long α 1,6-glucan chain. Non-typable *H. pylori* strains 147181, 147570 and 160708, containing different amounts of L-fucose, were selected for CE-MS analysis in the positive ion mode and the resulting spectra were compared to the CE-MS fragmentation pattern obtained for 26695 cells under the same conditions. Enzyme-treated and delipidated *H. pylori* cells from these strains were subjected to in-source collision-induced dissociation MS analysis (Li *et al.*, 2005; Wang *et al.*, 2005). This technique allows in-source fragmentation of the O-chain polysaccharide into repeating unit components, confirming its sequence. The resulting spectra were consistent with the presence of N-acetylglucosamine (LacNAc) repeating units, and fragment ions corresponding to LacNAc (*m/z* 366), LacNAc–N-acetylhexosamine (*m/z* 569) and double repeating units of LacNAc–LacNAc at *m/z* 731 were observed along with the fragment corresponding to the backbone core oligosaccharide (*m/z* 1244) (Fig. 2).

Table 2. Chemical analysis of LPS from non-typable strains of *H. pylori*

Approximate molar ratios of the alditol acetate derivatives are given. Analyses were performed on whole bacterial cells. Fuc, fucose; Glc, glucose; Gal, galactose; DD-Hep, DD-heptose; DL-Hep, DL-heptose.

Strain	Fuc	Glc	Gal	GlcNAc	DD-Hep	DL-Hep
32	0.2	4.0	2.0	2.4	1.5	1.0
40	0.2	2.0	2.0	1.3	6.0	1.0
71	0.2	2.1	1.2	1.9	1.5	1.0
72	0.2	2.0	1.6	2.0	1.4	1.0
77-2	0.2	1.0	0.7	2.2	1.4	1.0
77-5	0.3	2.0	1.4	1.8	1.8	1.0
146195-13	0.2	4.5	1.7	4.5	3.0	1.0
147181	0.1	1.0	1.3	2.2	2.2	1.0
147570	0.2	2.0	1.4	2.9	1.5	1.0
160708	0.3	5.3	2.2	4.0	2.0	1.0
26695	0.5	2.5	3.8	5.9	1.6	1.0

Analysis of Le^b-containing *H. pylori* isolates

Silver staining and Western blotting with anti-Le^b mAbs revealed significant variations in the O-chain length of Le^b-containing isolates (Fig. 3). In strains, 59, 79, 153085, 103, 108, 109, 147403 and 149489, the Le^b epitope was found to be co-expressed with Le^x and/or Le^y antigens (8/11 Le^b-containing isolates). Three Le^b-containing strains, namely, 79, 147403 and 149489, did not react in the Western blot format but were positive for the presence of Le^b antigen in WCE results. Two of these strains, 79 and 147403, had a low Le^b antigen content according to WCE results (Table 1). The high-molecular-mass ladder-like pattern produced by silver staining of the third strain, 149489, suggested the presence of a long α 1,6-glucan chain, in addition to Le^y and Le^b antigens, as corroborated by WCE data (Fig. 3a). Three Le^b-positive but Le^x- and Le^y-negative strains (99, 102 and 147570) reacted in the Western blot format with anti-Le^b mAbs and showed very diverse O-chain profiles capped by Le^b structures comprising a long O-chain in strain 99, an intermediate O-chain in strain 102 and a short O-chain in strain 147570 (Fig. 3b). Due to the limited number of Le^b-expressing cases, we were not able to produce any meaningful statistical analysis relating to potential correlation with data from cases of more severe histopathology or greater bacterial density.

Co-expression of Le^y and Le^x in more virulent strains of *H. pylori*

Co-expression of Le^x and Le^y antigens was observed in *H. pylori* strains possessing vacuolating-inducing VacA forms [16/50, 32 %, odds ratio (OR): 3.2, 95 % confidence interval (CI): 1.004–10.203] or functional T4SS (17/50, 34 %, OR: 3.886, 95 % CI: 1.191–12.681), or both (14/50, 28 %, OR: 3.800, 95 % CI: 1.159–12.456). However, no correlation was observed between the LPS content and the number of EPIYA phosphorylation motifs in the CagA protein (data not shown). Furthermore, no association was observed between the severity of histopathological findings (for chronic gastritis, $P=0.562$, Pearson's chi-squared=1.154; for chronic inflammatory infiltration, $P=0.427$, Pearson's chi-squared=1.704) or the levels of bacterial colonization in the antrum ($P=0.777$, Pearson's chi-squared=0.506) and the distribution of Le^x and Le^y antigens (data not shown). Finally, no statistical difference was observed in terms of histopathological features, especially bacterial density, between strains expressing type 1 (or non-typable) and type 2 Lewis antigens.

DISCUSSION

This study examined the expression of Le antigens and α 1,6-glucan in 50 *H. pylori* isolates from symptomatic Greek children. While the majority of the strains examined expressed type 2 Le^x and/or Le^y antigens (70 %), we showed that, unlike *H. pylori* strains isolated from adult population, paediatric isolates have the tendency for expression of the type 1 Le^b blood group antigen (22 %).

Fig. 1. Silver-stained SDS-PAGE (12.5% acrylamide) of proteinase K-treated whole cells of *H. pylori*.

In accordance with our previous findings (Altman *et al.*, 2008), in the majority of cases, the Le^b antigen was either co-expressed with type 2 Le^x and Le^y antigens or just with Le^y. Three Le^b-positive isolates were non-typable and produced LPS O-chains composed of LacNAc units capped

by Le^b antigens. Recent studies have demonstrated that *H. pylori* populations expressing Le^b were more efficient than non-Le^b expressers in colonizing Le^b-transgenic mice (Pohl *et al.*, 2009). In addition, it was shown that the *jhp562* gene, encoding β -1,3-galactosyltransferase, which is involved in

Fig. 2. CE-MS analysis of *H. pylori* cells in the positive ion mode using orifice voltage of 380 V. (a) Strain 26695 (control), (b) strain 147181, (c) strain 160708, (d) strain 147570.

Fig. 3. Silver-stained SDS-PAGE (12.5 % acrylamide) (a) and immunoblot (b) of proteinase K-treated whole cells of *H. pylori* Le^b-containing isolates. *H. pylori* strain SS1, negative control; LeB, Le^b-hexasaccharide-BSA, positive control.

the biosynthesis of type 1 Le^a and Le^b antigens, is associated with peptic ulcer disease (PUD) in Portuguese children (Oleastro *et al.*, 2010). Furthermore, a previous study of Taiwanese isolates of *H. pylori* provided evidence that the intensity of gastric Le^b and Le^x determinants could be correlated with bacterial density of *H. pylori* colonization in the stomach (Sheu *et al.*, 2003).

We did not find any correlation between histopathological findings or referral symptoms in children and the presence of the Le^b antigen. However, most of the strains examined in this study were from symptomatic children presenting with epigastralgia, vomiting and occasional oesophagitis and only two isolates were from children with PUD. Expression of Le^b in combination with Le^y was confirmed in both strains associated with PUD, in line with previous observations (Oleastro *et al.*, 2010). Four out of six *H. pylori* isolates from children experiencing vomiting were non-typable and one out of the six strains expressed Le^b exclusively. It has been previously shown that a higher intensity of gastric Le^b expression over the corpus could be correlated with an increased risk of ulcers in children (Yang *et al.*, 2008). More studies are needed to confirm the association between the expression of the Le^b phenotype and *H. pylori* infection in children.

It has been well established that 80 % of *cagA*-positive *H. pylori* strains express Le^x or Le^y and more than half of them express both (Heneghan *et al.*, 2000; Wirth *et al.*, 1996). Previous studies also suggested that the expression of type

2 Le antigens by *cagA*-positive isolates could counterbalance their proinflammatory effects (Wirth *et al.*, 1996). In a more recent study (Yahav *et al.*, 2000), it was shown that anti-CagA-seropositive *H. pylori*-infected children have a significantly higher incidence of persistent infection and harder-to-treat gastroduodenal disease. In contrast, in this study 15 of 35 strains expressing type 2 and/or type 1 Le antigens (30 %) were CagA-negative. Similar findings were previously reported by Gold *et al.* (2001) who observed the presence of marked gastritis in 27 % of *cagA*-negative *H. pylori* strains isolated from North American children. As previously suggested, loss of *cagPAI* and the prevalence of *cagA*- and T4SS-deficient isolates in children could be evolutionary and may offer a selective advantage during early colonization (Sgouras *et al.*, 2009).

Two pairs of strains examined in the present study, 146195-8 and 146195-13, and 105 and 160708, were isolated from children who underwent upper endoscopy, twice and at different ages. In both instances, the expression of Le antigens was associated with *H. pylori* isolates from younger children and disappeared with age. These results are consistent with previous observations that changes in gastric Le expression are different between adults and children and that the *H. pylori*-infected adults, but not the children, had a lower Le^b intensity over the antrum but higher intensity over the corpus than non-infected ones, as shown by immunohistochemical staining of gastric biopsies (Yang *et al.*, 2008). Furthermore, *H. pylori*-infected adults expressed higher

levels of Le^b and had a higher bacterial density in both antrum and corpus as compared with those with weak Le^b intensity (Yang *et al.*, 2008). Two of the examined strains, 77-2 and 77-5, represented different isolates from the same patient; both strains exhibited similar expression levels of α 1,6-glucan as determined by WCE and had similar LPS patterns as determined by silver staining, although they have been shown to express CagA protein with different numbers of EPIYA motifs (Sgouras *et al.*, 2009).

An unusually high frequency of non-typable isolates (30 %) was also observed. It was previously suggested that the lack of Le expression in *cagA*-positive isolates is uncommon (Wirth *et al.*, 1996). However, in our study, six out of 15 non-typable strains (40 %) were CagA-positive by WCE. Selected non-typable strains were subjected to further characterization by composition and CE-MS analyses, performed directly on bacterial cells. The results confirmed the presence of mainly LacNAc-containing structures in these isolates and low fucosylation levels. The prognostic significance of these findings remains to be established.

Another interesting feature of paediatric isolates of *H. pylori* is the overwhelming presence of α 1,6-glucan. We recently reported that 44.7 % of Chilean *H. pylori* isolates expressed α 1,6-glucan, a polymeric component of the outer core region of LPS (Harrison *et al.*, 2011). Co-expression of α 1,6-glucan with Le^x and/or Le^y antigens in the majority of *H. pylori* strains examined suggests the importance of this LPS component in the pathogenesis of *H. pylori*. We have previously shown that α 1,6-glucan affects the colonizing ability of *H. pylori* and is required for initial colonization of the murine stomach (Altman *et al.*, 2008). In this context, the presence of α 1,6-glucan in *H. pylori* strains isolated from children could be considered another important phenotypic characteristic facilitating successful colonization and contributing to the antigenic diversity of *H. pylori* LPS.

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