The LacdiNAc-Specific Adhesin LabA Mediates Adhesion of *Helicobacter pylori* to Human Gastric Mucosa

Yannick Rossez,1,3,4 Pierre Gosset,1,2,5 Ivo G. Boneca,6,10 Ana Magalhães,13 Chantal Ecobichon,3,10 Celso A. Reis,13,14 Caroline Cieniewski-Bernard,1,4 Marie Joncquel Chevalier Curt,1,3,5 Renaud Léonard,1,3,4 Emmanuel Maes,1,3,4 Brice Sperandio,11 Christian Slomianny,1,7 Philippe J. Sansonetti,11,12 Jean-Claude Michalski,1,3,4 and Catherine Robbe-Masselot1,3,4

1Univ Lille Nord de France; 2UCLille, 3USTL, UGSF, IFR 147, 4CNRS, UMR 8576, 5Groupe Hospitalier de l’Institut Catholique Lillois/Faculté Libre de Médecine, Lille, 6EA 4488, Laboratoire d’Activité Physique, Muscle et Santé, 7Laboratoire de Physiologie Cellulaire, INSERM U 1003, Université des Sciences et Technologies de Lille, Villeneuve d’Ascq, 8Service d’Anatomie Pathologie, 9Institut Pasteur, 10INSERM, Equipe Avenir, Groupe Biologie et génétique de la paroi bactérienne, 11Unité de Pathogénie Microbienne Moléculaire et Unité INSERM 788, Institut Pasteur, 12Chaire de Microbiologie et Maladies Infectieuses, Collège de France; 13Instituto de Patologia e Imunologia Molecular da Universidade do Porto, and 14Institute of Biomedical Sciences Abel Salazar and Medical Faculty of the University of Porto, Portugal

**Adhesion of *Helicobacter pylori* to the gastric mucosa is a necessary prerequisite for the pathogenesis of *H. pylori*-related diseases.** In this study, we investigated the GalNAcβ1-4GlcNAc motif (also known as N,N'-diacetyllactosamine [lacdiNAc]) carried by MUC5AC gastric mucins as the target for bacterial binding to the human gastric mucosa. The expression of LacdiNAc carried by gastric mucins was correlated with *H. pylori* localization, and all strains tested adhered significantly to this motif. Proteomic analysis and mutant construction allowed the identification of a yet uncharacterized bacterial adhesin, LabA, which specifically recognizes lacdiNAc. These findings unravel a target of adhesion for *H. pylori* in addition to moieties recognized by the well-characterized adhesins BabA and SabA. Localization of the LabA target, restricted to the gastric mucosa, suggests a plausible explanation for the tissue tropism of these bacteria. These results pave the way for the development of alternative strategies against *H. pylori* infection, using adherence inhibitors.

**Keywords.** lacdiNAc; *Helicobacter pylori*; tropism; gastric mucins; adhesin.

*Helicobacter pylori* are gram-negative bacteria that infect over half of the world’s population. The species was first identified in 1983 by Marshall and Warren in the gastric mucosa of a patient with chronic gastritis [1]. Most individuals acquire *H. pylori* in childhood. These bacteria induce a chronic inflammation of the underlying mucosa, which tends to persist indefinitely unless treated. In absence of treatment, these pathogens participate in the development of 3 clinically important upper gastrointestinal diseases: gastroduodenal ulcer, gastric carcinoma (except cancer of the gastric cardia), and mucosa-associated lymphoid tissue lymphoma [2]. The ability of *H. pylori* to cause disease depends on a variety of host, environmental, and bacterial factors.

*H. pylori* colonize the gastric mucosa by adhering to epithelial cells and the mucus layer lining the gastric epithelium. The mucus layer is a powerful barrier that protects tissues from mechanical damage, as well as chemical erosion from peptic and HCl. Together with water, its main components are large glycoproteins called mucins, which consist of a large linear protein core (apomucin) carrying hundreds of branched chains of oligosaccharides along its entire length. The extreme length of these molecules, together with their high degree of glycosylation (>50% of their total weight) and their negative charge, are major determinants of their...
ability to form gels. Also, mucins undertake receptor-ligand interactions and communicate information about external cell conditions through signal transduction [3].

Most H. pylori reside within the mucous gel layer of the stomach that covers the apical surface of the gastric epithelium, with approximately 20% of the population found to bind directly to gastric epithelial cells [4]. Different molecular mechanisms have been proposed to explain bacterial adherence to the gastric mucins and cells. Among them, 2 H. pylori adhesins, BabA and SabA, have been well characterized with respect to their target specificities. BabA (the blood group antigen-binding adhesin) is specific for fucosylated blood group antigens H-type 1 and Leb but also extends its spectrum of binding to terminal residues of blood group A and B glycan determinants [5–7]. SabA (the sialic-acid binding adhesin) binds the sialyl Lea and sialyl Leb antigens [8, 9]. Although BabA and SabA are the most prevalent adhesins studied so far, not all H. pylori strains express these adhesins functionally [10, 11]. This implies that other bacterial entities must be involved in adhesion of these bacteria to gastric mucins and cells. Possible candidates include H. pylori outer membrane proteins (OMPs), but because the family is extensive, their functional role continues to be investigated.

In this work, we have identified and characterized a bacterial adhesin of H. pylori that binds specifically the lacdiNAc motif located on MUC5AC mucins, which we have termed “LabA” (ie, lacdiNAc-binding adhesin). The role of LabA in H. pylori gastric tropism and the use of analogs or derivatives of lacdiNAc to inhibit bacterial adhesion and thus as novel tools for the treatment of H. pylori infection are discussed.

**METHODS**

**Human Samples and Mucin Preparation**

Gastric tissues from 32 individuals were collected by Dr Jacques Bara according to protocols approved by the French national ethics committee. A summary of the age, sex, and blood group status of the donors is given in Supplementary Table 1. Mucins were purified as described before [12].

**Release of Oligosaccharides From Mucin by Alkaline Borohydride Treatment**

The gastric mucins were subjected to β elimination under reductive conditions (ie, 0.1 M NaOH and 1 M NaBH₄ for 24 hours at 45°C).

**Fractionation of the Oligosaccharide Alditols by High-Performance Liquid Chromatography (HPLC)**

The mixture of oligosaccharides was subjected to fractionation by HPLC (Dionex Chromelone System, Sunnyvale, CA) on a primary amino-bonded silica column (Supelcosyl, LC-NH₂, 4.6 x 250 mm, Supelco, Bellefonte, CA). The column was equilibrated with the initial solvent, using a mixture of acetonitrile H₂O (ratio, 80:20 by volume) with a flow rate of 1 mL/minute. After the injection, a linear gradient was applied for 80 minutes to achieve a ratio of 40:60 by volume, which was kept as isocratic conditions for 20 minutes. A second HPLC step on a C18 reverse-phase column was conducted for the fraction containing the glycans of interest. Oligosaccharides were eluted with H₂O. Oligosaccharides were detected by UV spectroscopy at 200 nm, using an UVD 170U detector (Dionex).

**Analysis of Glycans by Nanoelectrospray Mass Spectrometry**

All analyses were performed on a Q-STAR Pulsar quadrupole time-of-flight (TOF) mass spectrometer (Applied Biosystems/ MDS Scix, Toronto, Canada) fitted with a nanoelectrospray ion source (Protana, Odense, Denmark), as described previously [13].

**Analysis of Glycans by Nuclear Magnetic Resonance (NMR) Spectroscopy**

Samples were repeatedly treated with ²H₂O (99.97% ²H atoms, Euriso-top, CEA, Saclay, France). Chemical shifts were expressed in parts per million and calibrated using internal acetone D6 (δ¹H 2.225 and δ¹³C 31.55 parts per million). Spectra were acquired at 300 K on a 9.4-T spectrometer (Bruker), where ¹H resonated at 400.33 MHz, and ¹³C resonated at 100.25 MHz. To better determine characteristic reporter groups, different spectra were recorded, such as ¹H-1D, ¹H-¹H COSY, ¹H-¹H-TOCSY, and ¹H-¹³C HSQC NMR spectra.

**Immunohistochemical Analysis**

Human biopsy specimens were formalin fixed and paraffin embedded. Sections with a thickness of 3 μm were prepared from paraffin blocks of formalin-fixed specimens. Heat pretreatment of the sections for 10 minutes was performed in citrate buffer (pH 4.0). Negative-control slides were incubated without primary antibodies. Sections were incubated overnight with primary antibodies against MUC5AC (1:100), MUC6 (1:10), or lacdiNAc (1:100). After washing, Alexa Fluor–conjugated anti-mouse (1:500) or anti-rabbit (1:500) secondary antibody was added as appropriate and incubated for 60 minutes. Slides were counterstained with DAPI.

For double-immunofluorescent analysis of intestinal metaplasia, samples were treated as described above for single-immunofluorescent labeling with MUC5AC antibody (CLH2, diluted 1:5), followed by incubation with a second primary antibody against MUC2 (PMH1, diluted 1:10) or lacdiNAc (diluted 1:100) overnight. After washing, sections were incubated with Texas red–conjugated goat anti-mouse (1:500) or anti-rabbit (1:500) secondary antibody was added as appropriate and incubated for 60 minutes. Slides were counterstained with DAPI.

For bright-field evaluation of lacdiNAc expression, the horse-radish peroxidase (HRP)–avidin-biotin complex method was used. Briefly, after deparaffination and rehydration, endogenous peroxidase activity was blocked with 3% H₂O₂ in methanol. Then samples were incubated with normal rabbit serum diluted...
1:5 in phosphate-buffered saline (PBS) containing 10% bovine serum albumin for 30 minutes. Sections were then incubated with anti-lacdiNAc (dilution, 1:100). The slides were subsequently washed in PBS and incubated for 30 minutes with biotinylated rabbit anti-mouse antibody (dilution, 1:200) before incubation with HRP-avidin-biotin complex (Vectastain Elite ABC kit, Burlingame, CA), according to the manufacturer’s recommendations. Staining was performed with 3,3′-diaminobenzidine tetrahydrochloride containing 0.02% hydrogen peroxide. Slides were counterstained with Mayer’s hematoxylin. Negative controls were performed by replacing primary antibody with PBS.

**Animal Tissue Samples**
Stomachs from 6–8-week-old C57Bl/6 mice and from 3-month-old Sprague Dawley rats were obtained according to animal experimentation guidelines. Every specimen was fixed in Carnoy’s solution and embedded in paraffin wax. Serial sections with a thickness of 3 µm were cut and used for immunohistochemical analysis.

**Bacterial Strains and Growth Media**
*H. pylori* strains B128 [15], 26695 [16], J99 [17], G27 [18], P12 [19], and B38 [20] were cultivated on blood agar plates or brain-heart infusion medium with 10% decomplemented fetal calf serum, with or without supplementation, with an antibiotic-antifungal mix (0.31 µg/mL of polymyxin B, 2.5 µg/mL of amphotericin B, 12.5 µg/mL of vancomycin, and 6.25 µg/mL of trimethoprim) and appropriated antibiotics (20 µg/mL of ampicillin, 20 µg/mL of kanamycin, 5 µg/mL of apramycin, or 5 µg/mL of chloramphenicol) at 37°C in a microaerophilic atmosphere.

**Assay of *H. pylori* Adhesion to Gastric Mucosa**
*H. pylori* was labeled with fluorescent isothiocyanate and adhesion assays were performed as previously described [14]. For inhibition assays, the bacteria (50 µL of the suspension containing 10⁸ bacteria/mL diluted 2.5-fold in protein-free blocking buffer) were preincubated 2 hours with lacdiNAc or Lewis b (50 µL of glycans at 1 mM). Evaluation of bacterial binding was estimated by determining the number of adherent bacteria under 400 × magnification. Each value represents the mean value for at least 5 different fields.

**Identification by Proteomic Analysis of LabA**
Bacterial lysates from B128 and 26695 strains were incubated overnight with or without free lacdiNAc glycan at 2 mM before co-incubation with human purified gastric mucins. After centrifugation (2655 g for 2 minutes) to pellet insoluble mucins and bound proteins, the supernatant was collected and proteins were separated on 7.5% gel electrophoresis. Differentially expressed proteins were submitted to “in gel” trypsin digestion. Protein identification was performed using peptide mass fingerprinting on a matrix-assisted laser desorption/ionization TOF/TOF mass spectrometer (AB Sciex TOF TOF 5800, Applied Biosystems, Foster City, CA). Proteins were identified using Protein Prospector (available at: http://prospectore.ucsf.edu/) from protein databases (UniProt release 2011_03 – 8 Mar 2011) and are numbered according to the HPB128155g82 peptide sequence, as follows: (1) aa45-GIQDLSDSYER-aa55, (2) aa72-QSADPNINNAR-aa83, (3) aa360-AQAEILNR-aa367, (4) aa514-TQELGSNPFR-aa523, (5) aa514-TQELGSNPFR-aa524, and (6) aa561-YYGFDYNHTYNK-aa573.

**Construction of the OMP Mutant**
The bank of the entire set of open reading frames of *H. pylori* strain 26695 was previously cloned into DH1 and inactivated systematically with the miniTn3-Km [21]. The oipA (hp0636) and hopD (hp0025) genes were inactivated using the miniTn3-Km strategy because these genes did not have any other downstream genes cotranscribed. Polymerase chain reaction was used to confirm the miniTn3-Km insertion.

**RESULTS**

**Immunolocalization of the LacdiNAc Motif Along the Gastrointestinal Tract**
The expression of lacdiNAc at the luminal surface of different human organs (stomach, colon, small intestine, salivary glands, and bronchial mucosa) was performed by immunohistochemical analysis. Double immunostaining was performed with polyclonal anti MUC5AC antibody (LUM5-1) and monoclonal anti MUC6 (CLH-5) or lacdiNAc antibodies (273-3F2). Among the human gastric tissues examined, the expression of lacdiNAc was absent in cardiac glands (Figure 1A), limited to the surface of the fundic mucosa (Figure 1B), and expressed more deeply in pyloric glands (Figure 1C). In the stomach body, lacdiNAc antibody stained the superficial and foveolar epithelium, with staining extending to the middle of the mucosal layer (Figure 1D). Where lacdiNAc was detected, it always colocalized with MUC5AC mucins. However, MUC5AC immunoreactivity continued deeper into all glands, indicating that this moiety was not always present on MUC5AC. No colocalization with MUC6 was detected either in the mucous cells of the neck zone or in the parietal cells, nor was there any detection of MUC5AC (Figure 1E). In humans, MUC5AC is also present in mucus from the vagina and lung, as well as in the stomach. However, lacdiNAc was only observed in association with MUC5AC in the stomach (data not shown). One exception was in the metaplasia of duodenal mucosa, which is of gastric origins (Figure 1F). This is medically relevant in the sense that *H. pylori* actually can colonize the duodenum with gastric metaplasia. Likewise, in the incomplete type of intestinal metaplasia, which can be colonized by *H. pylori* [22] and is characterized by the coexpression of gastric and intestinal mucins, lacdiNAc and MUC5AC coexpression was observed (Figure 2A). In
**Figure 1.** Immunohistochemical analyses of MUC5AC, MUC6, and lacdiNAc expression and localization in the human different sections of normal stomach. Double immunostaining for MUC5AC detected by LUM5-1 in red and lacdiNAc detected by 273-3F2 in green in the cardiac region (A), fundus (B), and pylorus (C). LacdiNAc was observed only in the fundus and pylorus, whereas MUC5AC was present in all stomach regions. Colocalization of MUC5AC and lacdiNAc was observed in the surface mucous cells, as seen in the detailed body mucosa gland (D). E, Merged images corresponding to the double immunolabeling for MUC5AC in red and MUC6 in green detected by CLH-5 in the body. Colocalization of MUC5AC and lacdiNAc was observed in gastric metaplasia of the duodenum (F).

**Figure 2.** LacdiNAc expression evaluation by double immunofluorescence analysis in complete and incomplete intestinal metaplasia. Double immunofluorescence analysis of MUC5AC (in red) and lacdiNAc (in green) expression. A, Intestinal metaplasia of the complete type showing absence of expression of both MUC5AC and lacdiNAc (arrowhead). Adjacent normal gastric mucosa shows both MUC5AC and lacdiNAc expression (arrow). B, Intestinal metaplasia of the incomplete type with coexpression of MUC5AC and MUC2 (data not shown) display MUC5AC and lacdiNAc coexpression (double arrowhead). Adjacent metaplastic gland of the complete type lack the expression of both MUC5AC and lacdiNAc (arrowhead).
contrast, the complete type of intestinal metaplasia of the gastric
mucosa, which is characterized by loss of expression of the gas-
tric mucins MUC1, MUC5AC, and MUC6 and by de novo ex-
pression of intestinal MUC2 mucin [23], is not colonized by
H. pylori [22]. No lacdiNAc expression was observed in these
corresponding samples (Figure 2B). Therefore, we hypothesize
that the lacdiNAc motif expressed on MUC5AC could play a
role in H. pylori colonization in humans.

To complete this work, expression of lacdiNAc was assessed in
stomachs from 3 rodent species known to be potentially colonized
by various Helicobacter species. In the gastric mucosa of mice and
rats (data not shown), the staining resembled that seen in the
human-derived tissue, with expression of lacdiNAc limited to
the superficial foveolar epithelium. In gerbils, lacdiNAc was also
expressed at the surface of the crypts. However lacdiNAc detec-
tion did not colocalize with MUC5AC, because this mucin type
was exclusively expressed at the bottom of the crypts (Figure 3).

A Unique lacdiNAc Containing O-glycan Mediates
Binding of H. pylori to Gastric Mucosa

The O-glycans carrying the lacdiNAc motif were isolated from
purified mucins to study the binding of H. pylori to lacdiNAc.
Gastric mucins from 32 healthy adults were isolated by a com-
bination of isopycnic density gradient centrifugation and gel
chromatography. Oligosaccharides were released by reductive
β elimination, and the mixture of O-glycans was fractionated
by HPLC (Supplementary Figure 1A). The fraction containing
the glycan of interest was submitted to a further fractionation
step on a hydrophobic column, allowing separation of the lac-
diNAc glycan from others (Supplementary Figure 1B). These
experiments not only provided a pure preparation of oligosac-
charides but also allowed the relative abundance of this glycan
to be determined in each human sample tested (Supplementary
Table 1). The glycan carrying lacdiNAc represented around 7%
of human adult gastric mucin O-glycans. Its presence in gastric
mucosa was recently described on the basis of mass spectrom-
etry data [24], and we confirmed its structure, Fucα1-2Galβ1-3
(GalNAcβ1-4GlcNAcβ1-6)GalNAcoli, by performing extensive
NMR analysis (Supplementary Figure 1C and 1D).

The binding specificities of 6 H. pylori strains to mucosa were
assessed in competition assays, using either soluble commer-
cially available glycoconjugates (lacdiNAc MP and Leb) or the
purified natural glycan carrying the lacdiNAc motif. Significant
inhibition of H. pylori binding by 0.5 mM of lacdiNAc contain-
ing soluble free oligosaccharides was observed for all tested
strains except B128. The levels of inhibition varied between
strains and ranged from 26.2% to 61.6% for commercial lacdi-
NAc, which increased to 28.3%–73.2% for the purified human
gastric oligosaccharide (Figure 4). Our data were in agreement
with previous studies for competitive inhibition by Leb [7, 8],
showing a strain-dependent variation in binding (Figure 4E).
For example, H. pylori 26695 adhered strongly to lacdiNAc gly-
coconjugates, and preincubation of the bacteria with lacdiNAc
completely abolished bacteria binding, whereas only a weak
inhibition was observed for Leb (Figure 4A–D).

In complementary experiments, sections of human gastric
mucosa were pretreated with monoclonal antibodies against
the Leb and the lacdiNAc moieties and further assessed for bac-
terial binding. As an example, data obtained with J99 showed an
approximately 65% reduction in bacterial binding when the gas-
tric tissue sections were preincubated with either anti Leb or anti
lacdiNAc (Figure 4F). As expected, no reduction in the binding
of strain 26695 could be observed when tissue sections were pre-
treated with anti Leb, whereas 70% of the binding was annull-
ated by anti-lacdiNAc antibody. Bearing in mind that strain
26695 does not express functional BabA and SabA adhesins,
it can be hypothesized that lacdiNAc is recognized by yet un-
characterized adhesins. Strong inhibition of adhesion (about
75%) is observed with strain B128 when gastric mucosa is pre-
incubated with monoclonal anti lacdiNAc or Leb antibodies.
This result seems to contradict the data obtained when bacteria
were preincubated with the free corresponding glycans at 0.5
mM (Figure 4B–D). However, about the same rate of inhibition
was observed when bacteria were preincubated with these gly-
cans at 25 mM (data not shown).

Figure 3. Evaluation of lacdiNAc expression in gerbil gastric mucosa.
Expression of lacdiNAc (in green) was observed in the superficial
foveolar (S) epithelium of the gerbil gastric mucosa but was not harbored by
MUC5AC (in red).

Purification and Identification of an Adhesin
With lacdiNAc Binding Specificity

To better understand how H. pylori adheres to gastric mucosa, we
attempted to identify and purify the adhesin of H. pylori involved

Figure 4. Comparison of the inhibition of adhesion of strains of H. pylori to
human gastric mucosa. (A) Binding of strain 26695 (BabA–) to human
gastric mucosa in the presence of soluble lacdiNAc (black line), Leb (red line),
and their mixture (blue line). (B) Binding of strain 26695 to human gastric
mucosa pretreated with monoclonal antibodies against Leb (black line),
anti-lacdiNAc (red line), and their mixture (blue line). (C) Binding of strain
B128 (BabA+) to human gastric mucosa in the presence of soluble lacdiNAc
(black line), Leb (red line), and their mixture (blue line). (D) Binding of strain
B128 to human gastric mucosa pretreated with monoclonal antibodies against
Leb (black line), anti-lacdiNAc (red line), and their mixture (blue line).
Figure 4. Inhibitions of bacterial binding in situ. *Helicobacter pylori* 26695 was overlayed on gastric tissue sections (original magnification × 400; A). Microscopy data presented are representative of 30 gastric tissue sections per condition, and diagrams show the mean values of the inhibition of adhesion observed for 5 fields of each of these 30 tissue sections. Pretreatment with commercially available lactoNac (LDN; B) and the gastric mucin O-glycan carrying LDN (Hum LDN; D) reduced bacterial binding to gastric mucosa, whereas Lewis b antigen (Leb; C) did not affect binding of this strain displaying a nonfunctional BabA. E, Preincubation with 0.5 mM of soluble glycans reduced bacterial binding to the gastric mucosa. The percentage of adherent bacteria corresponded to the number of bacteria adhering to the gastric mucosa after preincubation with soluble glycans relative to the number of bacteria adhering to the gastric mucosa without preincubation with soluble glycans, multiplied by 100. F, Gastric tissue sections were pretreated with monoclonal antibodies against the Leb and the LDN. The graphic shows the percentage of bacteria binding to the human gastric tissues.
Figure 5. Identification of the LabA adhesin. Purified gastric mucins were mixed with bacterial lysates with or without free lacdiNAc glycan (A and B). After overnight incubation followed by centrifugation, the supernatant containing bacterial proteins and free adhesins was collected. B, In competition experiments with excess free lacdiNAc, all LabA bound to free lacdiNAc (C) and was recovered only in supernatant. Insoluble mucins and adhesins linked to glycans were recovered in the pellet. The supernatant containing bacterial adhesins was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (D). Lane 1, bacterial lysate from *Helicobacter pylori* B128 strain; lane 2, bacterial lysate from *H. pylori* B128 strain plus free lacdiNAc glycan. One band at 77 kDa was clearly increased in lane 1. The band was excised for proteomic analysis (E). All 10 peptides (sequences in green and yellow) were aligned with the HPB128155g82 gene (ie, the 10 peptides matched). The peptide sequence of 6 of them (sequences in yellow) was confirmed by matrix-assisted laser desorption/ionization time of flight (TOF) mass spectrometry. Thus, results suggest that LabA corresponds to the HPB128155g82 gene/HP002529 gene encoding HopD protein. Abbreviation: LDN, lacdiNAc.
Figure 6. Inhibition of bacterial binding in situ with *Helicobacter pylori* B128 wild-type and ΔlabA strains. A and B. Genetic organization of the 2 genetic loci carrying *hopD* and *oipA*. The genes, intergenic regions and cassettes are to scale (500 bp correspond to 1 cm). The *hopD* and *oipA* genes were inactivated with the mini*Trn*3-km. Insertion occurred in both cases at around 300 base pairs from the start of the gene. C–F. Adhesion of *H. pylori* to the surface of human gastric mucosa. The wild-type *H. pylori* B128 (C) and mutant ΔlabA (D) strains were overlayed on gastric tissue sections. Pretreatment with free soluble lacdiNAc reduced binding of the wild-type B128 strain (E), whereas adhesion of mutant ΔlabA was not affected by a pretreatment with lacdiNAc (F). G and H. In the deeper region of the foveola, where MUC5AC but not lacdiNAc is expressed (G), the adhesion of mutant ΔlabA (H) is similar to those of wild-type strains.
in recognition of lacdiNAc. Bacterial lysates from *H. pylori* strains B128 and 26695 were incubated with pure gastric mucins in the presence or absence of free lacdiNAc glycan provided as a binding competitor (Figure 5A–C). Proteins left unbound to gastric mucins were separated by 7.5% SDS-PAGE. One protein, with a size of 77 kDa, was significantly more abundant in the competitive experiments, corresponding to the putative adhesin (Figure 5D). The band was excised from the gel for proteomic analysis. Ten peptides were identified on the basis of peptide mass, of which 6 were sequenced (Figure 5E). All of the sequenced peptides matched peptides encoded by HP0025 gene in strain 26695. This gene belongs to the large *hop* family of *H. pylori* OMP genes and was identified as hopD [25]. We renamed this protein “LabA” because of its binding properties.

**LabA Is Involved in *H. pylori* Binding to Gastric Mucosa In Vivo**

To assess functional binding of the LabA adhesin, we generated a *labA* deletion strain of *H. pylori* B128 (Figure 6A). In situ assays showed a strong reduction of binding of the *H. pylori*Δ*labA* strain to the surface of gastric mucosa, compared with the wild-type strain (Figure 6C and 6D). Moreover, preincubation of Δ*labA* with free lacdiNAc did not modify the binding (Figure 6E and 6F), in contrast to what was found with the wild-type strain. In further support of a specific interaction between LabA and lacdiNAc, adhesion of Δ*labA* (Figure 6G) was similar to that of the wild-type strain in the deeper gastric mucosa, where MUC5AC but not lacdiNAc is expressed (Figure 6H). To assess the specificity of this effect, a second deletion mutant strain of *H. pylori* B128 was generated for the gene *oipA* (denoted ΔoipA; Figure 6B). Indeed, *oipA* encodes an OMP characterized to play a role in *H. pylori* adhesion but for which the recognition motif has not yet been identified. Preincubation with lacdiNAc profoundly reduced the binding of the ΔoipA mutant to the gastric mucosa, in contrast to that seen with the Δ*labA* strain (Supplementary Figure 2).

**DISCUSSION**

*H. pylori* exhibits strict host and tissue specificity. It colonizes exclusively gastric tissues from humans and a few other species. Tropism of the bacteria for the stomach is unlikely to be explained by the strongly acidic conditions generated by the stomach; indeed, the area of gastric metaplasia in the duodenum, incomplete type of intestinal metaplasia, and gastric tissue in Meckel’s diverticulum are all exclusively colonized [26, 27]. BabA and SabA are 2 well-characterized adhesins of *H. pylori* that bind Le^b^ blood group antigens and sialylated Le^b^ or Le^a^ determinants, respectively. However these glycan motifs are not restricted to the gastric mucosa and are also found on mucins from the digestive tract. Therefore, functional binding of these adhesins cannot explain the restricted tropism of this microorganism. In this study, we have demonstrated that lacdiNAc is expressed at the surface of the gastric mucous cells of humans and several animal species and colocalizes with gastric MUC5AC (this result was also confirmed by structural analysis of the glycosylation pattern of purified mucins) and *H. pylori* colonization under physiological conditions. In gastric and intestinal pathologies, characterized by both expression of MUC5AC and *H. pylori* adhesion, strong expression of lacdiNAc was observed. In contrast, in organs devoid of *H. pylori* colonization, lacdiNAc oligosaccharides were never detected, whether or not these organs expressed MUC5AC. Previous studies have provided evidence that MUC5AC constitutes a specific receptor for the bacteria but stopped short of identifying which peptidic or glycosylated domain of the mucin is implicated in the binding of *H. pylori* [18]. From in situ inhibition assays, we have demonstrated a strong adhesion of the bacteria to lacdiNAc. We suggest that this interaction may underlie the previously unexplained tropism of the bacteria for gastric tissue and its colocalization with MUC5AC. We have shown that the lacdiNAc-carrying glycan is exclusively expressed on gastric mucins and strongly inhibits *H. pylori* adherence in situ. This may explain the specific and restricted tropism of *H. pylori* for gastric mucosa. We have identified LabA as the *H. pylori* adhesin involved in the recognition of the lacdiNAc motif. Understanding the regulation of expression of lacdiNAc in physiopathological conditions will give information about the role played by the glycan in gastric homeostasis and its evolutionary significance.

As antibiotic resistance associated with *H. pylori* eradication is increasingly observed, there is a pressing need to develop alternative strategies [29]. Our discovery offers the possibility for new therapeutic alternatives to antibiotics to eradicate or control of *H. pylori* based on inhibition of bacterial adhesion. Since it is possible to chemically synthesize lacdiNAc, the present study provides a basis for development of analogs or derivatives of this glycan motif to treat *H. pylori* infection.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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