Introduction

*Helicobacter pylori* was the first bacterium classified as a type I carcinogen (maximum level) [1]. Since its discovery, many research projects have focused on virulence factors or genetic markers but few studies have included *H. pylori* strains associated with gastric mucosa associated lymphoid tissue (MALT) lymphoma [2–5]. Gastric lymphoma is considered to be the classic lymphoma of MALT-type of the digestive tract [6]. It is a B lymphoma with a very unusual pathogenesis and evolution which evolves very progressively and stays localized in the stomach for a long time. The development of the lymphoma is directly linked to the *H. pylori* infection although it is not known why this evolution occurs in only a very small number of infected subjects. A large number of molecular events participating in the lymphomagenesis of MALT lymphomas have already been described, in which a chronic antigenic stimulation plays a pivotal role. Moreover, as the incidence of MALT lymphoma may correlate with different inflammatory cytokines and gene polymorphisms, the role of the host immune response has not been clearly defined yet [7].

The role of *H. pylori* strains on B-cell proliferation in low-grade MALT lymphoma is well established [8–10]. However, some questions remained unanswered. What is the nature of the *H. pylori* antigens recognized by lymphocytes? How does this recognition occur? Dendritic cells (DC) could play a role in antigen recognition and in inflammatory response in this disease. Indeed, the response of DC to a specific organism depends on the pathways activated in response to the microbial agent and to the cells present in the environment [11]. *H. pylori* lipopolysaccharide (LPS) is one of the key effectors of Toll Like Receptor-4 and it has been shown that the nature of the Lewis-type antigens expressed on the surface of the LPS of *H. pylori* determines the interaction with DC via the recognition by a C-type lectin called DC-SIGN at the surface of DC [12]. It has been also suggested that interaction with DC-SIGN could influence the pro-inflammatory response. Lewis negative strains escape binding to DC and induce a strong Th1-cell response. In contrast, *H. pylori* strains that express Le^a^ and/or Le^b^ can bind to DC-SIGN and enhance the production of IL-10 which promotes a Th2-cell response and blocking of Th1-cell activation.

In the context of gastric MALT lymphoma, the chronicity of *H. pylori* infection is believed to be crucial [6]. According to Suarez et al., the chronic microbial antigen stimulation observed during persisting *H. pylori* infection constitutes an antigenic source in autoimmunity which leads to sustained B-cell stimulation, thus favoring lymphoid transformation and lymphoma development [13]. Therefore, in this chronic disease a molecular mimicry, which implies the expression of microbial pathogen motifs shared
with the host, could be mandatory to favor *H. pylori* persistence. Lewis determinants resemble autoantigens because of the molecular mimicry with the fucosylated Lewis antigens [14] expressed by the mucosal chief and parietal cells of the gastric glands as well as on the surface and foveolar epithelia [15].

Le\(^{\alpha}\) and Le\(^{\beta}\) (type 2 carbohydrates) are the dominant Lewis antigens in *H. pylori* LPS expressed by 90–90% of clinical isolates whereas Le\(^{\epsilon}\), Le\(^{\kappa}\), H-1 (type 1 carbohydrates), Lewis c, and sialyl Le\(^{\alpha}\) are rarely expressed (less than 5%) [16,17]. Three fucosyltransferases FutA, FutB and FutC are involved in Lewis antigen synthesis. FutA and FutB which have an \(\alpha_1,3\) and/or \(\alpha_1,4\)-fucosyltransferase activity are required for Le\(^{\alpha}\) or Le\(^{\kappa}\) antigen synthesis, respectively. FutC which has an \(\alpha_1,2\)-fucosyltransferase activity transfers an additional fucose to produce Le\(^{\epsilon}\) or Le\(^{\beta}\) antigens. The three corresponding genes, ie, futA (HP0379), futB (HP0651) and futC (HP0093-94), contain poly-C tracts at the 5' end that regulates their expression by a slipped strand mispairing mechanism [18].

The Le\(^{\epsilon}\) and Le\(^{\beta}\) antigens expressed on the LPS of *H. pylori* gastric MALT lymphoma strains has not yet been investigated. Therefore, the aim of this project was to study these LPS structures of *H. pylori* gastric MALT lymphoma strains in comparison to strains solely associated with chronic gastritis.

### Results

**Description of the cag pathogenicity island (cagPAI) status and vaculocating cytotoxin gene A (vacA) genotypes of the strains included in the present study**

As indicated in material and methods, 40 MALT strains (19 cagPAI (−) and 21 cagPAI (+)) and 39 gastritis strains (17 cagPAI (−) and 22 cagPAI (+)) were included in this study.

The 39 gastritis strains were classified according to the signal region as either s1 or s2 or according to the middle region as m1 or m2 of the vacA gene. The distribution of the different alleles, s1m1, s1m2 and s2m2 of the gastritis strains were 16 (41%), 15 (38.5%) and 8 (20.5%), respectively.

As previously determined [4], the different vacA alleles for MALT strains were 11 s1m1 (27.5%), 12 s1m2 (30%) and 17 s2m2 (42.5%).

**Distribution of the Le\(^{\epsilon}\) and Le\(^{\beta}\) antigens among the strains according to the disease status and virulence factors**

Le\(^{\epsilon}\) was identified in 21 MALT strains (52.5%) and Le\(^{\beta}\) in 30 strains (75%). Le\(^{\epsilon}\) was identified in 29 gastritis strains (74.3%) and Le\(^{\beta}\) in 31 strains (79.5%). Depending on the Lewis antigens expressed, the strains were divided into four groups. Five gastritis strains (12.8%) and 5 MALT strains (12.5%) were Le\(^{\epsilon/\beta}\) negative. Three gastritis strains (7.7%) and 5 MALT strains (12.5%) only expressed Le\(^{\epsilon}\). Five gastritis strains (12.8%) and 14 MALT strains (35%) solely expressed Le\(^{\beta}\). Finally, 26 gastritis strains (66.7%) and 16 MALT strains (40%) were both Le\(^{\epsilon/\beta}\) positive.

There was a significant association between cagPAI status and Le\(^{\beta}\) expression among MALT strains (p<0.0001), but not in gastritis strains (p = 0.64, NS).

There was a significant interaction between Lewis antigens and cagPAI status in relation to the disease status (P value for interaction = 0.028), therefore we stratified the analysis.

**Table 1. Distribution of the Lewis phenotypes among gastritis and MALT lymphoma strains of Helicobacter pylori according to the cagPAI status.**

| Lewis antigens | Gastritis (n (%)) | MALT (n (%)) | Odds ratio * (95% confidence interval) |
|---------------|------------------|--------------|---------------------------------------|
| Among cagPAI (−) |                  |              |                                       |
| Le\(^{\epsilon/\beta}\) | 11 (64.7) | 1 (5.3) | 1.0 (reference) |
| Le\(^{\epsilon/\beta}\) | 1 (5.9) | 2 (10.5) | 19.9 (0.8–485.0) |
| Le\(^{\beta}\) | 2 (11.8) | 12 (63.2) | **64.2** (4.9–841.0) |
| Le\(^{\epsilon}\) | 3 (17.6) | 4 (21.0) | 12.9 (0.9–178.2) |
| Among cagPAI (+) |                  |              |                                       |
| Le\(^{\epsilon/\beta}\) | 15 (68.2) | 15 (71.4) | 1.0 (reference) |
| Le\(^{\epsilon/\beta}\) | 2 (9.1) | 3 (14.3) | 2.7 (0.20–37.7) |
| Le\(^{\beta}\) | 3 (13.6) | 2 (9.5) | 0.69 (0.08–5.6) |
| Le\(^{\epsilon}\) | 2 (9.1) | 1 (4.8) | 0.39 (0.03–5.8) |

*Odds of being MALT/odds of being gastritis, with adjustment for gender and age.

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Le\(^{\epsilon}\) and Le\(^{\beta}\) were associated with MALT with an odds ratio (OR) of 64.2 with 95% confidence intervals (95% CIs) 4.9–841.0 when compared to Le\(^{\epsilon/\beta}\) positive strains (Table 1).

Among cagPAI positive strains, Le\(^{\epsilon}\) were expressed in 17 gastritis strains (73.3%) and 19 MALT strains (85.7%); and Le\(^{\beta}\) in 18 gastritis strains (81.8%) and 17 MALT strains (81.0%). cagPAI positive strains expressing solely Le\(^{\epsilon}\) were not associated with MALT when compared to Le\(^{\epsilon/\beta}\) positive strains (OR 0.4; 95% CI 0.03–5.8) (Table 1).

vacA genotypes did not modify the association between Lewis antigens and disease status.

**Sequencing of futA and futB for Le\(^{\epsilon/\beta}\) negative strains**

The LPS extraction was verified on SDS/PAGE gels after silver staining (data not shown) for the Le\(^{\epsilon/\beta}\) negative strains: 5 gastritis strains (3 cagPAI (−) and 2 cagPAI (+)) (G152, G171, G541, G32 andG447) and 5 MALT lymphoma strains (3 cagPAI (−) and 1 cagPAI (+)) (M30, M33, M40 and M48). The futA and futB genes were then sequenced. According to the number of C repeats that regulates the on/off status of the genes, 9 strains were futA “off” and one strain futA “on” (M54), 8 strains were futB “off” and 2 strains futB “on” (G447, G541). To summarize, a total of 7 strains were “off” for both the futA and futB genes (ie, M30, M33, M40, M48, G152, G171 and G32), one strain was futA “on” only (M54) and in two strains only futB was “on” (ie, G341 and G447) (Table 2). The number of C repeats varied between 8 to 13 for futA and from 8 to 11 for futB. The first 91 bp of the 5’ part of the genes which contains the CG repeats, as well as the deduced amino acid sequenced, are shown in Table 2.

**Discussion**

The LPS expressed by *H. pylori* strains has been suggested to be important for gastric colonization, adherence and immune evasion through a “camouflage” mechanism in order to escape the host immune response [19]. The influence of Lewis antigens is also believed to participate in the polarization of the Th1/Th2 inflammatory response. Lewis antigens expressed by *H. pylori* MALT strains could play a key role in the physiopathology of the disease. We previously described the main virulence factors...
**Table 2.** Signal-sequence coding region of the *futA* and *futB* genes and deduced amino acid sequences of 10 Le\(^{xy}\) negative *Helicobacter pylori* strains.

| Loci | Strains | Sequence of the signal-peptide coding region | C repeat | Status | Deduced amino acid sequence |
|------|---------|-----------------------------------------------|----------|--------|-----------------------------|
| *futA* | M48 | ATGTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 8 | Off | MFQPLLDAFIESASIEKMASKSPPPKNRCGELVGR* |
| M30 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMASKSPPP* |
| M33 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| M40 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| G152 | ATGTTTCCGGCCCCTACTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- GGGTTTCTAAATCTCCCCCCCCC-- TAAAAATCGCTG | 9 | Off | MFRLDNFGKASFEKRVSKPPP* |
| G171 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| G541 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| M32 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 11 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| G447 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 11 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| M54 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 13 | On | MFQPLLDAFIESASIEKMKMSKLPPPPLKIAVANWGD |

| *futB* | M30 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 8 | Off | MFQPLLDAFIESASIEKITSKSPPLKNRCGELVGR* |
| M48 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 8 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| M33 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| M40 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| M54 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| G152 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| G171 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 9 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |
| G447 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 10 | On | MFQPLLDAFIESASIEKMKMSKLPPPPLKIAVANWGD |
| G541 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 10 | On | MFQPLLDAFIESASIEKMKMSKLPPPPLKIAVANWGD |
| G32 | ATGTTTCCAGCCCTTACCTAGACGCTTTATAGAAGGCGCTTCATTTGAAAAA- TGGCCTCAATATCCTCCCCCCC- -- -- -- TAACATCGCTG | 11 | Off | MFQPLLDAFIESASIEKMKMSKLPPP* |

G: gastritis strain. M: MALT strain. The stop codon is indicated in bold type for strains having the “off” status (except for strains M48, G32 for the *futA* locus, and M30, M48, M33 and G32 for the *futB* locus) and the end of the protein is indicated by an asterisk.

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expressed by gastric MALT lymphoma strains except the Lewis antigens [4]. Therefore, in the present study, the Lewis antigen expression was compared between H. pylori MALT strains and the chronic gastritis strains. The gastritis strains were selected on the presence or absence of the cagPAI in order to match the gastric MALT lymphoma strains and to constitute two comparable groups.

Most of the strains (59/69, 85.5%) included in the present study expressed at least one Lewis antigen (Le⁺ and/or Le⁻) which is in line with previous reports [17]. Although it is well known that a combination of all several methods (serodot, Western blotting and enzyme-linked immunosorbent assay) may allow a more accurate assessment of Le expression [20], only 10 double Le⁺/y⁻ negative strains were identified and mainly among cagPAI negative strains (80%) as already described. Concerning these double negative strains and according to the number of C repeats, the correlation with the futA and futB sequences was not perfect. Indeed, because 3 of these 10 strains were “on” for futA or futB, we cannot exclude that they do not express some of the other less common Lewis antigens [16].

The Lewis antigen patterns were then analyzed according to the cagPAI status and vacA genotypes of the strains. This is an issue that has been extensively discussed previously. A first study published by Wirth et al. showed that Le⁺ expression in H. pylori was correlated with its CagA status [21]. Some studies suggest that such a correlation does not exist [22] or that an inverse correlation exists between CagA positive strains and Le⁺ expression [23]. Brouet et al. studied the LPS of 155 isolates from atrophic gastritis patients, and identified two main clusters of strains, those which were CagA positive and double Le⁺/y⁻ positive, and a second cluster comprised of CagA negative and solely Le⁺ positive or double Le⁺/y⁻ negative [24]. The authors also showed a correlation with vacA genotypes. However, more recently, Skoglund et al., considering only Le antigen expression, were not able to confirm such a correlation for atrophic gastritis isolates [25]. Nevertheless, CagA positive isolates are believed to be more aggressive and more exposed to the immune system. Therefore, a camouflage by an efficient mimicry process could help them to persist in the stomach of their host. A similar association was found in the present study for Le⁺ and cagPAI positive gastric MALT lymphoma strains. Indeed, the proinflammatory properties of cagPAI positive gastric MALT lymphoma strains have been previously proven using the AGS cell model (most cagPAI positive MALT lymphoma strains harbored a functional cagPAI) but no important proinflammatory factors could be identified in cagPAI negative H. pylori MALT strains [26]. According to Moran, all of the discrepancies between Lewis antigens expression and cagPAI status are believed to be due to the adaptation of H. pylori strains to different populations [19,27]. However, in contrary to the study of Brouet et al., the present study did not identify a correlation between Lewis antigens and vacA genotypes [24].

Concerning the disease status among cagPAI negative gastric MALT strains, a significant association was found with Le⁺. Although we were not able to quantify the amount of Le⁺ expressed by the strains, the Le⁺ bands obtained by Western Blot were very intense (data not shown). It has been suggested that a high expression of Le⁺ could mask the Le⁺ epitopes [18]. The activity of futC in the synthesis is very critical. Indeed, strains that have a relatively high level of futC activity could transform nearly all of the Le⁺ giving rise to a phenotype expressing solely Le⁺, whereas relatively lower activity is believed to be responsible for a simultaneous Le⁺ and Le⁻ phenotype. Like futA and futB, futC is regulated by a slipped-strand mispairing mechanism through a polyG tract as well as an imperfect TAA repeat in the mid-region of futC [28].

The futC expression could also be influenced by translational frameshift, transcriptional regulation of certain genes as well as deletion in the promoter region. Therefore, we believe that the high Le⁺ expression in cagPAI negative MALT strains should be studied more extensively on a genetic basis.

What could be the role of Le⁺ in cagPAI negative MALT strains? During H. pylori chronic infection, Le⁺ expression has been implicated in the pathogenesis of atrophic gastritis through the induction of autoreactive antibodies against the gastric mucosa [19]. For example, such antibodies have been found in patients with atrophic gastritis and gastric cancer. Skoglund et al. recently published a paper in which they showed that the HPAG1 strain (which is a Le⁺ positive strain) was able to switch in vitro to both Le⁺ and Le⁻ expression, while the same strain in a mouse model of gastric atrophy remained to exclusively express Le⁺. The authors concluded that the switch in Lewis antigen expression was linked to different gastric environments, and possibly gastric pH. However, the authors were not able to reproduce this phenomenon in vitro by using culture broth at different pH [25]. Indeed, it has been shown that futC transcription can be regulated by environmental factors such as gastric acid and host immunity [29]. The study of Skoglund et al. indicates that Lewis expression at the time of isolation can be considered as a “snap-shot” of a strain at a particular time of the disease status/development reflecting the bacterial adaptation to in vivo conditions. Therefore, the higher expression of Le⁺ in cagPAI negative MALT strains identified in the present study raises the question of the presence of a component of atrophy in patients infected by these types of strains. This particular point has been suggested in long-term infected BALB/c mice [30,31]. The presence of auto anti Le⁺ antibodies in the physiopathology of this disease should also be evaluated.

In the context of gastric MALT lymphoma, intra Th lymphocytes are essentially Th0, and Greiner et al. showed the important role of cytokines produced by Th2 lymphocytes (IL-4, IL-10) [32]. Similarly, animal models for MALT lymphoma (BALB/c mouse) showed a Th2 type response [31,33]. Because most of the MALT strains expressed at least one Lewis antigen, they should be able to polarize a Th2 inflammatory response and to address this major point an in vitro study is currently being performed using DCs.

Our study shows for the first time that gastric MALT lymphoma strains, especially cagPAI negative, can be distinguished from gastritis strains. This particular Lewis profile is probably representative of the adaptation of H. pylori during the micro-environmental changes that the bacterium encounters in the course of infection. Moreover, it could represent an adaptive mechanism to the host response that should be explored in vivo in a murine model of gastric MALT lymphoma, and especially on a long-term survival in the host and modulation of host immune responses (autoimmune antibody production, release of cytokines).

Materials and Methods

Bacterial strains

A subset of the French gastric MALT lymphoma strain collection provided by the French National Reference Center for Campylobacters and Helicobacters was included in this study: 40 strains from gastric MALT lymphoma patients (24 men and 16 women), mean age 58.4 (13.8)). For all these strains the cagPAI status and vacA genotypes have been previously published: 19 cagPAI (−) and 21 cagPAI (+) [4].

A selection of 39 strains isolated from chronic gastritis only patients (16 men and 23 women, mean age 48.3 (13.2)) were included as a population control. All of these strains were isolated from patients included in the Swedish Kalixanda study which was...
performed on a general adult population from northern Sweden. The strains included in this study were selected according to their \textit{cag}PAI status (17 \textit{cag}PAI (−) and 22 \textit{cag}PAI (+)) in order to be comparable with the MALT strain collection [34].

LPS purification

All of the strains (previously obtained from single colonies) were defrosted on G plates before LPS extraction. Then \textit{H. pylori} was grown in bruccella broth supplemented with 5% fetal bovine serum and 1% IsoVitaleX Enrichment to exponential phase (OD$_{600nm}$ between 0.3 and 0.6). An equal amount of bacteria from each strain was harvested by centrifugation and washed once in PBS. The LPS from each bacterial pellet were extracted twice using a hot phenol protocol as already described [18]. After an overnight precipitation in ethanol and sodium acetate, the LPS were dried in air and resuspended in water.

Polyacrylamide gel electrophoresis and immunoblotting

LPS extracts were analyzed by SDS/PAGE by using a 4–15% separating polyacrylamide gel and blotted onto a polyvinylidene difluoride membrane (Immun-Blot PVDF Membrane, Biorad, Hercules, CA). Mouse anti-Le$^a$ (MCA 1313, Serotec, dilution 1/500) and anti-Le$^b$ (BG-8, Signet Laboratories Inc., Dedham, MA, dilution 1/2000) were used as primary antibodies and horseradish peroxidase-conjugated goat anti-mouse IgM (Start 86P, Serotec, dilution 1/1000) as a secondary antibody. Membranes were incubated with antibodies and developed with enhanced chemiluminescence (ECL) (Amersham Pharmacia Biosciences, Buckinghamshire, UK) as already described [18,25,35]. The LPS extraction for Le$^a$ and Le$^b$ negative strains was also verified on SDS/PAGE gels stained with silver.

PCR and sequencing

\textit{H. pylori} DNAs were purified with DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations from bacterial pellets obtained from the same bruccella broth as those used for LPS extraction. For gastritis strains, the \textit{vacA} alleles (s and m regions) were detected by PCR as previously described [4,36]. Finally, the 5’end parts of genes encoding FutA and FutB were amplified and sequenced for Le$^a$ and Le$^b$ negative gastritis and MALT strains identified in this study. The sequences of the primers used for futA and futB amplification and sequencing are indicated in Table 3.

| Primer | Sequence 5’-3’ | Target use |
|--------|----------------|------------|
| futA-F | CTCTCGTGTATCTCTGGCTATT | futA$^a$ | PCR and sequencing |
| futA-R | AGATGACGTCGATCAGATA | futA$^a$ | PCR and sequencing |
| futB-F | GCCCTAATCAAGCCTTGTTG | futB$^b$ | PCR and sequencing |
| futB-R | AAAACACACGCTGCAAAAA | futB$^b$ | PCR and sequencing |
| futA-futB-F | TCTCACACCTTCTTCCTCC | futA and futB$^b$ | Sequencing |
| futA-futB-R | TCTCACACTTCTTCCTCCC | futA and futB$^b$ | Sequencing |

$^1$indicates that the primer binding sequence is located outside of the coding region of the gene.

$^2$indicates that the primer binding sequence is located inside of the coding region of the gene. 

F for a forward primer; R for a reverse primer.

References

1. IARC (1994) Schistosomes, liver flukes and \textit{Helicobacter pylori} in “Monographs on the evaluation of carcinogenic risks to humans” IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994 61: 1–241.
2. Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, et al. (1999) Clinical relevance of the \textit{Helicobacter pylori} gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A 96: 12770–12773.
3. Koehler CI, Moes MB, Diens HP, Kriegsmann J, Schirmacher P, et al. (2003) \textit{Helicobacter pylori} genotyping in gastric adenocarcinoma and MALT lymphoma by multiplex PCR analyses of paraffin wax embedded tissues. Mol Pathol 56: 36–42.
4. Lehours P, Menard A, Dupouy S, Bergey B, Richy F, et al. (2004) Evaluation of the association of nine \textit{Helicobacter pylori} virulence factors with strains involved in low-grade gastric mucosa-associated lymphoid tissue lymphoma. Infect Immun 72: 800–888.
5. Lehours P, Dupouy S, Bergey B, Ruskone-Foumestraux A, Delchier JC, et al. (2004) Identification of a genetic marker of \textit{Helicobacter pylori} strains involved in gastric extramucosal marginal zone B-cell lymphoma of the MALT-type. Gut 53: 951–957.
6. Lehours P, Megraud F, (1997) T helper 1 effector cells specific for \textit{Helicobacter pylori} in the gastric antrum of patients with peptic ulcer disease. J Immunol 158: 962–967.
7. D’Ellios MM, Manghetti M, De Carli M, Costa F, Baldari CT, et al. (1997) \textit{Helicobacter pylori} virulence factors with strains involved in gastric MALT lymphomas. Gastroenterology 112: 1105–1112.
8. Rescigno M, Lopatin U, Chieppa M (2008) Interactions among dendritic cells, macrophages, and epithelial cells in the gut: implications for immune tolerance.Curr Opin Immunol 20: 669–675.
9. Bergman MP, Earing A, Smits HH, van Vliet SJ, van Bodegraven AA, et al. (2004) \textit{Helicobacter pylori} modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. J Exp Med 200: 979–990.
10. Suarez F, Lorholary O, Hermine O, Lecuit M (2006) Infection-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation. Blood. 107: 3034–3044.
11. Vandenbreucke-Gravas CM, Appelmelk BJ (1989) \textit{Helicobacter pylori} LPS: molecular mimicry with the host and role in autoimmunity. Ital J Gastroenterol Hepatol 30 Suppl 3: S259–S260.
12. Kobayashi K, Sakamoto J, Kito T, Yamamura Y, Koshikawa T, et al. (1993) Lewis blood group-related antigen expression in normal gastric epithelium, intestinal metaplasia, gastric adenoma, and gastric carcinoma. Am J Gastroenterol 88: 919–924.

Statistical methods

All analyses were conducted using the SAS 9.2 package (SAS Institute, Cary, NC). Correlations between genes were tested by the Spearman method. ORs with 95% CIs derived from unconditional logistic models were used to assess associations between Lewis antigens and disease status, and \textit{vacA} genotypes and disease status, with adjustments for gender and age. Interaction effects between and among genotypes and Lewis antigens were tested by inclusion of product terms in regression models.

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Author Contributions

Conceived and designed the experiments: PL. AS. Performed the experiments: PL. Analyzed the data: PL. ZZ. Contributed reagents/materials/analysis tools: PL. FM. LE. Wrote the paper: PL. AS. FM. LE.
16. Monteiro MA, Chan KHN, Rasko DA, Taylor DE, Zheng PY, et al. (1998) Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J Biol Chem 273: 11533–11543.

17. Heneghan MA, McCarthy CF, Moran AP (2000) Relationship of blood group determinants on *Helicobacter pylori* lipopolysaccharide with host Lewis phenotype and inflammatory response. Infect Immun 68: 937–941.

18. Nilsson C, Skoglund A, Moran AP, Annuh H, Enstrund L, et al. (2006) An enzymatic ruler modulates Lewis antigen glycosylation of *Helicobacter pylori* LPS during persistent infection. Proc Natl Acad Sci U S A 103: 2963–2968.

19. Moran AP (2000) Relevance of fucosylation and Lewis antigen expression in the bacterial gastroesophageal pathogen *Helicobacter pylori*. Carbohydr Res 345: 1952–1965.

20. Wirth HP, Yang M, Karita M, Blaser MJ (1996) Expression of the human cell surface glycoconjugates Lewis x and Lewis y by *Helicobacter pylori* isolates is related to cagA status. Infect Immun 64: 4598–4605.

21. Marshall DG, Hynes SO, Coleman DC, O'Morain CA, Smyth CJ, et al. (1999) Lack of a relationship between Lewis antigen expression and cagA, CagA, vacA and VacA status of Irish *Helicobacter pylori* isolates. FEMS Immunol Med Microbiol 25: 79–90.

22. Gonzalez-Valencia G, Munoz-Perez L, Morales-Espinosa R, Camorlinga-Ponce M, Munoz O, et al. (2008) Lewis antigen expression by *Helicobacter pylori* strains colonizing different regions of the stomach of individual patients. J Clin Microbiol 46: 2783–2785.

23. Broitman N, Moran A, Hynes S, Sacaravitch C, Menegald F (2002) Lewis antigen expression and other pathogenic factors in the presence of atrophic chronic gastritis in a European population. J Infect Dis 185: 503–512.

24. Skoglund A, Backhed HK, Nilsson C, Bjorkholm B, Normark S, et al. (2009) A changing gastric environment leads to adaptation of lipopolysaccharide variants in *Helicobacter pylori* populations during colonization. PLoS One 4: e5885.

25. Broutet N, Moran A, Hynes S, Sakarovitch C, Megraud F (2002) Lewis antigen expression and other pathogenic factors in the presence of atrophic chronic gastritis in a European population. J Infect Dis 185: 503–512.

26. Atherton JC, Cao P, Peck RM, Tummuru MKR, Blaser MJ, et al. (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori* - Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 270: 17771–17777.