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Helicobacter pylori adhesion to gastric epithelial cells is mediated by glycan receptors

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Helicobacter pylori adhesion to gastric epithelial cells is mediated by glycan receptors

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Abstract

Helicobacter pylori adhesion to gastric epithelial cells constitutes a key step in the establishment of a successful infection of the gastric mucosa. The high representation of outer membrane proteins in the bacterial genome suggests the relevance of those proteins in the establishment of profitable interactions with the host gastric cells. Gastric epithelial cells are protected by a mucous layer gel, mainly consisting of the MUC5AC and MUC6 mucins. In addition to this protective role, mucins harbor glycan-rich domains that constitute preferential binding sites of many pathogens. In this article, we review the main players in the process of *H. pylori* adhesion to gastric epithelial cells, which contribute decisively to the high prevalence and chronicity of *H. pylori* infection. The BabA adhesin recognizes both H-type 1 and Lewis b blood-group antigens expressed on normal gastric mucosa of secretor individuals, contributing to the initial steps of infection. Upon colonization, persistent infection induces an inflammatory response with concomitant expression of sialylated antigens. The SabA adhesin mediates *H. pylori* binding to inflamed gastric mucosa by recognizing sialyl-Lewis a and sialyl-Lewis x antigens. The expression of the BabA and SabA adhesins is tightly regulated, permitting the bacteria to rapidly adapt to the changes of glycosylation of the host gastric mucosa that occur during infection, as well as to escape from the inflammatory response. The growing knowledge of the interactions between the bacterial adhesins and the host receptors will contribute to the design of alternative strategies for eradication of the infection.

Key words: *Helicobacter pylori*; Bacterial adhesins; Lewis antigens; Glycan receptors; Chronic gastritis; Gastric carcinogenesis

Helicobacter pylori and gastric carcinogenesis

Helicobacter pylori is a Gram-negative spiral-shaped microaerophilic bacterium specialized in the colonization of the human stomach, that infects more than a half of the world's population (1). Although most infected individuals show no clinical symptoms, *H. pylori* can cause gastric ulcers and persistent infection may cause chronic atrophic gastritis with the development of intestinal metaplasia (IM), dysplasia and gastric carcinoma (2). Gastric adenocarcinoma is the second cause of cancer death worldwide. In 1994, based on epidemiologic evidence, the International Agency for Research on Cancer classified *H. pylori* as a class I carcinogenic agent. The crucial role of *H. pylori* in the carcinogenic pathway was further confirmed using various animal models, which demonstrated that *H. pylori* infection induced gastritis, IM and gastric carcinoma (3,4). The development of

disease upon infection depends on bacterial virulence factors, host susceptibility features and other environmental factors such as smoking and diet. Among the bacterial virulence factors, the CagA protein encoded within the cytotoxin-associated gene pathogenicity island (cagPAI) and the vacuolating cytotoxin VacA have been demonstrated to be important features in determining the clinical outcome of *H. pylori* infection (5). In addition, genetic polymorphisms in genes codifying for host inflammatory cytokines, including IL-1B, TNF-A, IL-10, and IL-8, as well as in genes participating in the innate immune response such as TLR4, confer an increased risk of gastric cancer and its precursor lesions development in the presence of *H. pylori* (6).

Mucins and their role in the host-pathogen interplay

Mucins are heavily glycosylated high molecular weight

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glycoproteins synthesized by several secretory epithelial cells. Mucins can be produced either as membrane-bound or secreted products and constitute the major component of the mucous viscous gels that lubricate and act as a physical barrier, providing protection for epithelial cells that line the respiratory and gastrointestinal tracts and form the ductal surfaces of organs such as the breast, pancreas and kidney (7).

The human mucin (MUC) family includes 21 members (MUC1 to MUC21) with a common structural feature: a tandem repeat domain comprising sequences of amino acids repeated in tandem, which are rich in proline, threonine and serine residues, constituting the PTS (Pro/Thr/Ser) domains. These domains are extensively glycosylated through GalNAc O-linkages at the serine and threonine residues (7).

In a healthy gastric mucosa, the mucins produced include MUC1, MUC5AC and MUC6. The membrane-associated MUC1 is expressed in foveolar cells and, to a lesser extent, in mucous glands. The secreted MUC5AC mucin is restricted to the foveolar epithelium and is a major constituent of the surface mucous gel layer, whereas the expression of the secreted MUC6 is limited to the glands (8-10). This mucin distribution determines the gastric glycosylation pattern since expression of MUC5AC is accompanied by similar distribution of fucosyltransferases leading to co-expression of type 1 Lewis a (Le^a) and Lewis b (Le^b) blood group antigens, while MUC6 expression is associated with the type 2 Lewis x (Le^x) and Lewis y (Le^y) antigens (Figure 1) (11).

H. pylori is mainly found within the gastric mucous layer and rarely colonizes deeper portions of the gastric mucosa. This distribution has been explained based on the production of mucins carrying carbohydrate chains with terminal α 1,4-GlcNAc residues attached to core2-branched O-glycans by the gland's mucous cells (12,13). *In vitro* experiments show that α 1,4-GlcNAc-capped O-glycans function as a natural antibiotic by inhibiting the biosynthesis of a major cell wall component and thus hampering *H. pylori* growth (13). Recently, it has been demonstrated that the membrane-associated MUC1 can limit *H. pylori* binding to gastric epithelial cells both by steric inhibition of binding to other cell surface ligands and by acting as a releasable decoy (14). These observations are in agreement with previous studies showing that mice deficient in Muc1 were more susceptible to *H. pylori* infection (15).

Chronic gastritis occasionally evolves to IM, characterized by the aberrant expression of MUC2 and the sialyl-Tn antigen, which are markers normally expressed in intestinal mucosa (12,16). Two distinct profiles of mucin expression have been described in IM, one corresponding to the complete type characterized by decreased expression of MUC1, MUC5AC and MUC6 and *de novo* expression of MUC2, and the other corresponding to the incomplete type characterized by the co-expression of the typical gastric mucins MUC1, MUC5AC and MUC6 with the intestinal

MUC2 (16). Complete IM is not colonized by *H. pylori* but a few cases of incomplete IM with *H. pylori* colonization have been described, suggesting that infection is dependent on the gastric microenvironment, which is largely determined by the mucin and carbohydrate composition of the gastric mucin layer (10,17).

***Helicobacter pylori* adhesion to human gastric mucosa**

H. pylori colonizes the gastric mucosa by adhering to the mucous epithelial cells and the mucous layer lining the gastric epithelium. Adhesion to the gastric cells is a crucial step in the establishment of a successful infection because it provides protection from clearance mechanisms such as liquid flow, peristaltic movements or shedding of the mucous layer. Approximately 4% of the *H. pylori* coding potential encodes a diverse repertoire of outer membrane proteins including the *Helicobacter outer* membrane porins (Hop) and Hop-related protein (Hor) subfamilies. The currently identified adhesins are phylogenetically clustered in the Hop subfamily and display a substantial degree of homology to each other (18,19).

Blood group antigen-mediated *Helicobacter pylori* adhesion

In the early 90's, *in vitro* adhesion assays identified the fucosylated blood group antigens H-type 1 and Le^b as mediators of *H. pylori* adhesion to human gastric epithelial cells (20). Later the Le^b-binding adhesin, named blood group antigen-binding adhesin (BabA), was identified and purified by receptor activity-directed affinity tagging (Figure 2). Two corresponding genes encoding BabA have been cloned: babA1 and babA2, but only the babA2 gene was shown to be functionally active (21). *H. pylori* strains expressing the BabA adhesin were shown to bind to the human gastric MUC5AC from healthy individuals in a Lewis b-dependent manner (22,23).

The bacterial Lewis b-binding phenotype is epidemiologically associated with the presence of the *cagPAI* (21,24). Several studies have established the clinical relevance of the babA2 gene, suggesting it as a marker to identify patients at higher risk for specific *H. pylori*-related diseases (24,25). Considering the clinical relevance of BabA, a vaccine strategy based on this adhesin might be used to target virulent strains of *H. pylori* (21,26).

H. pylori strains are able to adapt their outer membrane expression profile according to alterations in host environment, including changes in mucosal glycosylation patterns, by switching on and off gene expression. The presence of highly homologous genes allows allelic replacement between genomic areas with different transcriptional activity. It has been demonstrated *in vitro* that if the babA2 gene is disrupted the bacteria can regain the Le^b-binding phenotype

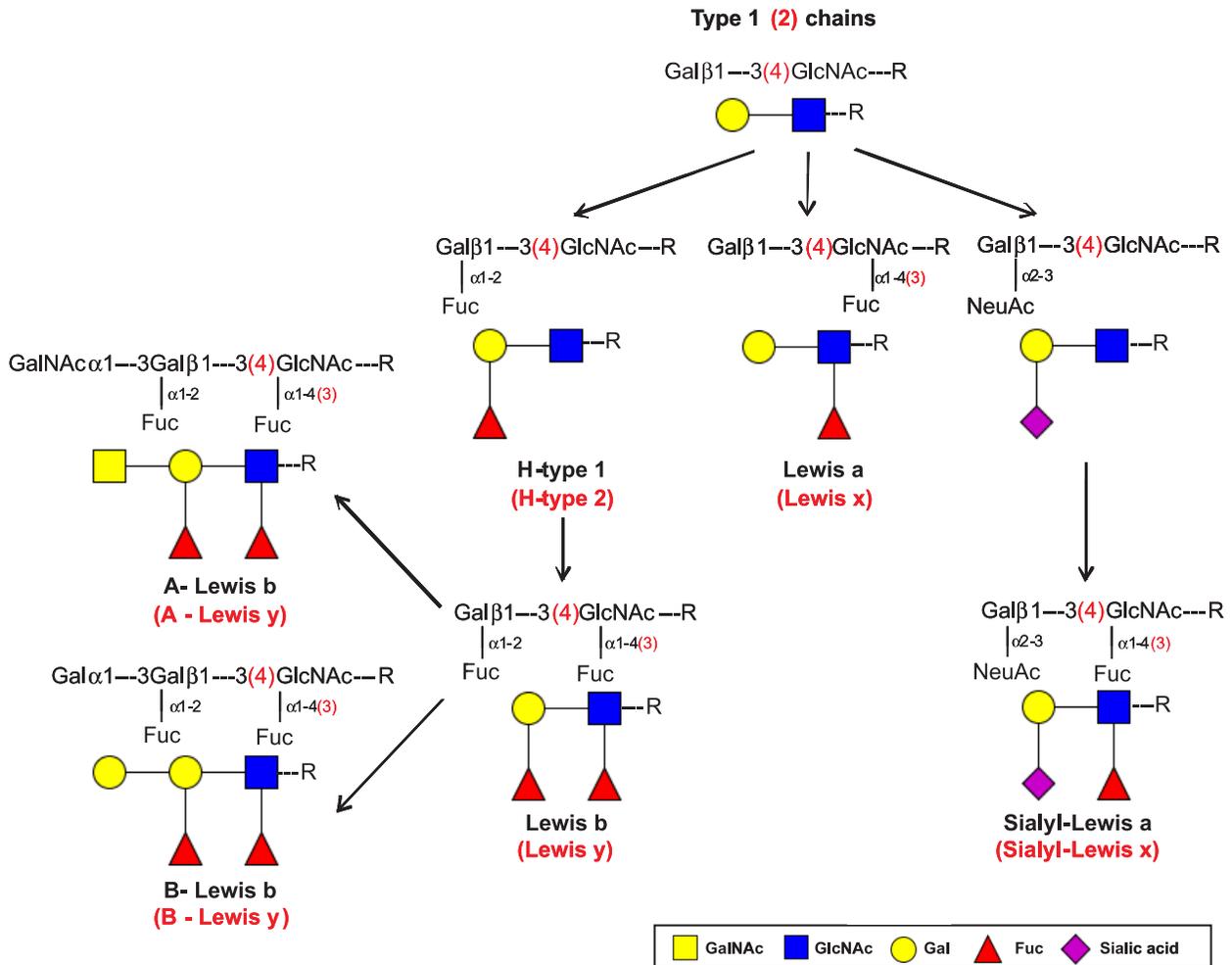


Figure 1. Schematic representation of the terminal ABH structures and Lewis epitopes present in O-glycans. Type 1 chains are characterized by the Galβ1,3GlcNAc linkage (represented in black), while type 2 chains display a Galβ1,4GlcNAc linkage (represented between parentheses and in red).

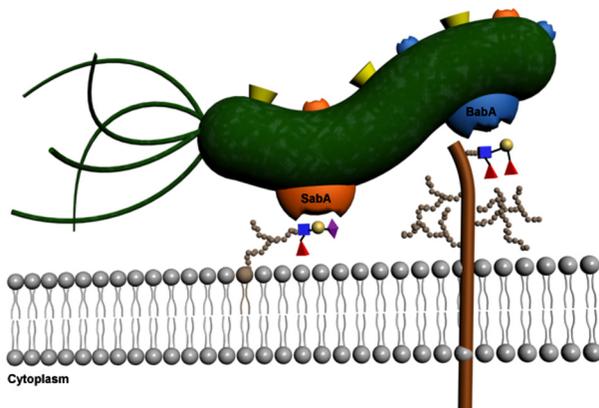


Figure 2. Glycan-mediated adhesion of *Helicobacter pylori* to gastric cells. The figure illustrates some of the characterized bacterial-glycan interactions that contribute to a successful colonization of the gastric mucosa. *H. pylori* BabA mediates binding to either Lewis b or H-type 1 structures (not represented in the figure) present in secreted or membrane-associated glycoproteins expressed on the gastric mucosa of healthy secretor individuals, while SabA recognizes the inflammation-induced sialylated antigens, sialyl-Lewis a and sialyl-Lewis x expressed on glycoproteins and glycolipids. Although the host receptors have not yet been determined, several other bacterial adhesins (represented in yellow) may contribute to bacterial binding to gastric epithelial cells.

by recombining the silent *babA1* gene into the *babB* locus where it is then expressed, encoding a functionally active adhesin (27). Moreover, reisolated strains from Rhesus macaques infected with a BabA-positive strain lost their Lewis b-binding capacity by switching off *babA* expression either by slipped-strand mispairing (SSM) or by recombination of the *babB* gene into the *babA* locus (28). Recently, these observations have been extended to different animal hosts, including mice and gerbils, and six amino acid changes in the BabA sequence have been identified as sufficient to abolish the Le^b-binding phenotype (29). BabA metastability and heterogeneity in Le^b antigen binding contributes to bacterial persistence, allowing periodic activation and deactivation of virulence adequate to the host response to infection (27,30). The plasticity of BabA adhesin has also been illustrated by the finding that some *H. pylori* strains are generalists, which means that they are able to tolerate the GalNAc (blood group-A) or Gal (blood group-B) terminal structures and bind A-Le^b and B-Le^b in addition to Le^b, while other strains, so-called specialists, exclusively bind to naked Le^b and a few strains exclusively bind to A-Le^b (Figure 1) (31). More recently, *H. pylori* strains, which express BabA adhesin but are unable to bind any Le^b structure, have been described (32).

The fucosylated H-type 1 and Le^b antigens are naturally expressed on the gastric mucosa of secretor and Lewis-positive individuals. The secretor status depends on an active FUT2 enzyme, an $\alpha(1,2)$ fucosyltransferase that catalyzes the addition of terminal $\alpha(1,2)$ fucose residues (33). Inactivating mutations in the secretor gene affect 20% of the human population and have been associated with reduced susceptibility to infections by Norwalk virus and *H. pylori* (34-37). We have used an animal model of non-secretors, the Fut2-null mice, to characterize the glycosylation profile and evaluate the effect of the observed glycan expression modifications in *H. pylori* adhesion. As expected, Fut2-null mice showed marked alteration in gastric mucosa glycosylation, characterized by diminished expression of $\alpha(1,2)$ fucosylated structures. This altered glycosylation profile resulted in the absence of Fuca(1,2)-dependent binding of calicivirus virus-like particles. Regarding *H. pylori* adhesion, we observed that strains expressing a functional BabA adhesin showed decreased adhesion to the gastric mucosa of Fut2-null mice whereas the binding pattern of strains that only express the sialic acid-binding adhesin SabA was not altered, demonstrating that this impaired adhesion could be strictly attributed to reduced expression of BabA ligands in the gastric mucosa of Fut2-null mice (38).

***Helicobacter pylori* adhesion during persistent infection**

Persistent *H. pylori* colonization of the gastric mucosa results in inflammation with concomitant expression of sialylated glycans such as sialyl-Le^a and sialyl-Le^x (Figures 1

and 2) (39,40). Parallel to what was described for humans, in the Rhesus monkey model, *H. pylori* infection resulted in an increase of sialylated mucosal antigens and a concomitant decrease in fucosylated antigens (41). Similar to BabA, the sialic acid-binding adhesin, SabA, has been identified by the retagging technique based on its affinity for sialyl-Le^x (39). The minimal structure required for SabA adhesin binding was shown to be NeuAc α 2-3Gal (42,43). Accordingly, it has been demonstrated that SabA interaction with the host gastric sialyl-Le^x antigen enhances *H. pylori* colonization in patients with weak or no Le^b expression (44).

Recently, we have demonstrated that *H. pylori* infection induces several alterations in the glycosylation-related gene expression profile of a human gastric cell line. Interestingly, the observed gene expression modifications were highly related to the degree of pathogenicity of the infecting *H. pylori* strain (45). Among the genes that were up-regulated by *H. pylori*, a specific glycosyltransferase, β 3GlcNAcT5, was identified and this enzyme was reported to be involved in the biosynthesis pathway of carbohydrate chains such as sialyl-Le^x on glycolipids. The induction of β 3GlcNAcT5 expression was specific of the highly pathogenic *cagPAI*-positive strains. *In vitro* experiments demonstrated that overexpression of the β 3GlcNAcT5 enzyme led to an up-regulation of sialyl-Le^x expression, concomitant with increased *H. pylori* SabA-mediated adhesion to gastric cells (45).

As previously described for the *babA* gene, *sabA* gene expression can be modulated by phase variation through SSM. The length of a dinucleotide cytosine-thymine repeat tract near the 5' end of the open reading frame determines if translation results in a truncated non-functional protein or in a full-length functional adhesin (39,46). In addition to SSM, SabA protein production is also controlled at the transcriptional level by the acid-responsive signal (ArsRS) two-component signal transduction regulatory system (46). This capacity of rapidly switching on and off BabA and SabA expression allows a continuous adaptation of the bacterial binding properties to the glycan profile modifications that occur during the inflammation process. This adaptation is essential for the maintenance of a chronic infection.

Other players that participate in the *Helicobacter pylori* adhesion process

Although BabA and SabA are the most prominent adhesins studied in detail so far, it is important to note that not every *H. pylori* strain expresses functional BabA or SabA adhesins, suggesting that other bacterial proteins are involved in *H. pylori* adhesion to gastric cells. It has been demonstrated that the adherence-associated lipoprotein A and B (AlpA and AlpB) and HopZ participate in *H. pylori* binding, but its corresponding host receptors remain to be determined (19,47,48). Additionally, two sulfo-binding *H. pylori* proteins have been described, the neutrophil-

activating protein (NAP), which binds specifically to sulfated oligosaccharide structures such as sulfo-Le^a, sulfogalactose and sulfo-N-acetyl-glucosamine on mucins, and Hsp70 that has been suggested to mediate sulfatide recognition under stress conditions (49,50). Heparan sulfate glycosaminoglycans have also been described to have a role in *H. pylori* adhesion to cell-line models (51,52). *In vitro* studies have shown that *H. pylori* infection up-regulates expression of syndecan-4, which represents one of the major sources of heparan-sulfate on the cell surface (53,54). These results were further validated *in vivo* by demonstrating that *H. pylori*-infected individuals expressed syndecan-4 in the foveolar epithelium of the gastric mucosa and that this expression was dependent on the *cagPAI* status of the infecting strain (53). Although several studies have favored a role for heparan sulfate glycosaminoglycans in the process of bacterial adhesion to epithelial cells there is still no explanation of how the induction of syndecan-4 by highly pathogenic *H. pylori* strains can contribute to the higher aggressiveness of these strains.

***Helicobacter pylori* mimics the host Lewis antigens**

Lipopolysaccharide (LPS) is a structural component of the outer cell wall of all Gram-negative bacteria. LPS is composed of a long-chain fatty acid anchor called lipid A, a core sugar chain, and a variable carbohydrate chain named O antigen. In contrast to several other pathogens, *H. pylori* LPS is of very low toxicity to the host. Interestingly, the O antigen of *H. pylori* shares structural homology with the Lewis blood group antigens expressed in human gastric mucosa, including antigens based on both type 1 chains, namely Le^a and H-type 1, and type 2 chains, like Le^x and Le^y (Figure 1) (55). The expression of these antigens undergoes phase variation, with different bacterial fucosyltransferases being switched on and off, contributing to a dynamic glycosylation even in the same host. It has been proposed that Le^x structures expressed by the *H. pylori* O antigen side-chain may promote adhesion to gastric epithelial cells, but the relevance of this interaction as well as the nature of the Le^x-receptors in gastric mucosa remain unknown (56,57). Beside the potential role in bacterial adhesion, this antigen mimicry may contribute to immune tolerance towards bacterial antigens (58). Furthermore, this mimicry process can induce the production of autoantibodies that recognize the host gastric epithelial cells, favoring the development of autoimmune-associated disease (58).

Strategies to inhibit *Helicobacter pylori* binding to human gastric mucosa

The increasing antibiotic resistance associated with *H. pylori* eradication by antimicrobial therapy is raising the need to search for alternative strategies such as those

based on blocking bacterial adhesion to host receptors. A strategy of carbohydrate-dependent inhibition of *H. pylori* colonization using porcine milk has been tested with success in mice, suggesting that milk from certain pig breeds may have a therapeutic and or prophylactic effect on *H. pylori* infection (59). In addition, ongoing studies are addressing the potential of using synthetic carbohydrates to inhibit *H. pylori* adhesion to gastric epithelial cells.

A human domain antibody specific for BabA adhesin has been recently isolated and proved to efficiently inhibit Le^b binding and to prevent adhesion of BabA-expressing *H. pylori* strains to human gastric mucosa (60). These experiments suggest its potential therapeutic application in combination with Le^b glycoconjugates in the treatment of antibiotic-resistant *H. pylori* infection.

Final remarks

Although *H. pylori* levels of infection are decreasing, the number of infected individuals is still very high, with half of the world population estimated to be infected. Antibiotic-based eradication therapies should be carefully considered due to the observed increased antibiotic resistance. Knowing that infection and persistent colonization of human gastric mucosa by *H. pylori* is highly dependent on bacterial adhesion to gastric epithelial cells, an effort should be made in order to better understand which bacterial lectin-host glycan interactions are truly critical for adhesion. Mucin type-O glycans have been demonstrated to be crucial players in the interaction between the bacteria and host gastric epithelial cells. The BabA adhesin recognizes both Le^b and H-type 1 structures expressed by the gastric epithelial cells of healthy secretors, while SabA mediates adhesion to inflamed gastric mucosa by binding the sialylated sialyl-Le^a and sialyl-Le^x antigens (Figure 2). However, some *H. pylori* strains express neither BabA nor SabA adhesins, which implies that other outer membrane proteins, already described or still unidentified, are participating in the adhesion process.

The clarification of why only a small percentage of *H. pylori*-infected individuals develop disease remains a major puzzling question regarding *H. pylori* infection. Besides the current knowledge of host genetic polymorphisms, bacterial virulence features and environmental factors that contribute to the clinical outcome of *H. pylori* infection, it is expected that the diversity of carbohydrate expression on gastric mucosa among individuals may confer distinct susceptibilities to *H. pylori* colonization and infection. This is demonstrated by the reduced susceptibility of non-secretor individuals to become infected by BabA-expressing *H. pylori* strains.

The characterization of the glycan receptors essential for *H. pylori* adhesion will allow the identification of individuals at higher risk to develop *H. pylori* infection-associated diseases, as well as the design of new infection eradication strategies based on inhibition of bacterial adhesion to gastric epithelial cells.

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