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REVIEW

Roles of gastric mucin-type *O*-glycans in the pathogenesis of *Helicobacter pylori* infection

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Helicobacter pylori **is a Gram-negative bacterium that infects over 50% of the world's population. This organism causes various gastric diseases such as chronic gastritis, peptic ulcer, and gastric cancer. *H. pylori* possesses lipopolysaccharides that share structural similarity to Lewis blood group antigens in gastric mucosa. Such antigenic mimicry could result in immune tolerance against antigens of this pathogen. On the other hand, *H. pylori* colonizes gastric mucosa by utilizing adhesins that bind Lewis blood group antigen-related carbohydrates expressed on gastric epithelial cells. After colonization, *H. pylori* induces acute inflammatory responses mainly by neutrophils. This acute phase is gradually replaced by a chronic inflammatory response. In chronic gastritis, lymphocytes infiltrate the lamina propria, and such infiltration is facilitated by the interaction between L-selectin on lymphocytes and peripheral lymph node addressin (PNAd), which contains 6-sulfo sialyl Lewis X-capped *O*-glycans, on high endothelial venule (HEV)-like vessels. *H. pylori* barely colonizes gland mucous cell-derived mucin where α1,4-GlcNAc-capped *O*-glycans exist. In vitro experiments show that α1,4-GlcNAc-capped *O*-glycans function as a natural antibiotic to inhibit *H. pylori* growth. These findings show that distinct sets of carbohydrates expressed in the stomach are closely associated with pathogenesis and prevention of *H. pylori*-related diseases, providing therapeutic potentialities based on specific carbohydrate modulation.**

Keywords: 6-sulfo sialyl Lewis X-capped *O*-glycan/α1, 4-GlcNAc-capped *O*-glycan/cholesterol α-glucoside/ cholesterol α-glucosyltransferase/*Helicobacter pylori*

Introduction

Impact of Helicobacter pylori discovery

Spiral microorganisms in the stomach had been observed in the 1930s and 1940s (Doenges 1938; Freedburg and Barron 1940), but little attention was paid to gastric microorganisms. In 1983, Marshall and Warren first isolated and succeeded in culturing the bacterium *Helicobacter pylori* from the gastric mucosa of patients with chronic gastritis (Warren and Marshall 1983; Marshall and Warren 1984). As a heroic act, Marshall himself drank a culture of *H. pylori* to prove that the bacteria could infect a healthy person and cause gastritis (Marshall et al. 1985). Their epoch-making discovery revealed that *H. pylori* is associated with various gastric diseases such as chronic gastritis, peptic ulcer, and malignant tumors including gastric carcinoma and malignant lymphoma, and the eradication of this microorganism likely prevents such gastric disorders (Rauws and Tytgat 1990; Montalban et al. 1995; Fukase et al. 2008). For their achievement, Marshall and Warren won the Nobel Prize in Physiology or Medicine in 2005 (Megraud 2005).

Specialized traits of H. pylori

H. pylori is a spiral-shaped, Gram-negative, and microaerophilic bacterium, measuring approximately 2.5–5.0 μm in length. *H. pylori* is a member of a genus of bacteria that have adapted to the ecological niche provided by gastric mucus, where there is little competition from other microorganisms (Liu and Crawford 2005). Many specialized traits allow this organism to flourish in the harsh environment of the stomach. First, *H. pylori* elaborates a large amount of urease which produces ammonia and carbon dioxide resulting from hydrolysis of endogenous urea, thereby buffering gastric acid in the immediate vicinity of the organism. *H. pylori* also possesses numerous long flagella, and the flailing movements of the flagella allow them to swim through viscous gastric mucus. Finally, *H. pylori* have adhesins that enhance adhesion with gastric epithelial cells by recognizing specific carbohydrate structures, such as the Lewis b blood group antigen and glycolipids having sialyl dimeric Lewis X (Ilver et al. 1998).

Epidemiology of H. pylori infection

H. pylori infection occurs worldwide and affects over 50% of the world's population, but the prevalence of infection varies greatly from country to country. The overall prevalence is highly correlated with socioeconomic status measured by household crowding and parental income (Hopkins et al. 1990; The EUROGAST Study Group 1993). Prevalence among adults is 70–90% in many developing countries and 25–50% in industrialized countries (Farinha and Gascoyne 2005).

The mode of transmission has not yet been fully defined; however, it is widely believed that the organism is transmitted directly from person to person by premasticated foods (oral–oral spread) or gastric contents (gastric–oral spread) (Dunn et al. 1997). It is now generally accepted that most individuals acquire *H. pylori* infection in childhood (Kumagai et al. 1998). Once the

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stomach is colonized by *H. pylori* and left untreated, the organism persists for decades, if not for a lifetime (Everhart 2000).

H. pylori-associated gastric diseases

Chronic Gastritis. Following *H. pylori* infection, a chronic, usually lifelong mucosal inflammation (gastritis), develops with concomitant appearance of serological responses against the bacterium. However, *H. pylori* is resistant to innate and acquired immune responses, and the immune system fails to remove the organism effectively (Sipponen and Hyvarinen 1993). Chronic gastritis leads eventually to mucosal atrophy characterized by a decrease in the proper gastric glands.

Peptic Ulcer. Peptic ulcers are chronic, often solitary lesions that occur in gastroduodenal mucosa exposed to aggressive action of acid-peptic juices. These lesions appear to be produced by an imbalance between mucosal defense mechanisms and damaging forces. The pathogenesis of peptic ulcers appears to be multifactorial, and the apparent role of *H. pylori* in peptic ulcers cannot be overemphasized. However, *H. pylori* infection is present in virtually all patients with duodenal ulcers and about 70% of those with gastric ulcers. Furthermore, antibiotic treatment of *H. pylori* infection promotes healing of ulcers and tends to prevent their recurrence (Liu and Crawford 2005).

Gastric Adenocarcinoma. Gastric adenocarcinoma is the fourth most common cancer and the second leading cause of cancer-related death worldwide (Parkin 2001). Gastric adenocarcinoma can be divided into two distinct histological subtypes (Lauren 1965), each with different epidemiological and clinicopathological features. One subtype is intestinal-type adenocarcinoma, which usually occurs at a later age, and progresses through a relatively well-defined series of histological steps, namely, chronic gastritis (associated with *H. pylori* infection), atrophy of pyloric glands, intestinal metaplasia, and dysplasia (Sipponen and Marshall 2000). The other subtype is diffuse-type adenocarcinoma, which more commonly affects younger people and is not associated with intestinal metaplasia (Sipponen and Marshall 2000).

Intestinal metaplasia, which is marked by the replacement of gastric epithelial cells with other epithelial cells such as columnar absorptive cells and goblet cells of intestinal morphology (Sipponen et al. 1985) (Figure 1), has been categorized into two major types: one is the complete type, which is characterized by the presence of absorptive cells, Paneth cells, and goblet cells secreting sialomucins and corresponds to the small intestine phenotype, and the other is the incomplete type, which is characterized by the presence of columnar and goblet cells secreting sialo and/or sulfomucins (Reis et al. 1999). These two types of intestinal metaplasia can also be distinguished by altered mucin expression patterns. While the intestinal mucin MUC2 is expressed in goblet cells of both types of intestinal metaplasia (normal gastric mucosa does not express MUC2 (Reis et al. 1997)), MUC5AC and/or MUC6 are expressed in the incomplete type but not in the complete type (Reis et al. 2000).

In spite of the fact that *H. pylori* has been categorized as a carcinogen, screening for and treatment of infected individuals to prevent gastric adenocarcinoma is not generally accepted (Fock et al. 2008). However, recently, Fukase and colleagues (2008) from the Japan Gast Study Group revealed that eradication of

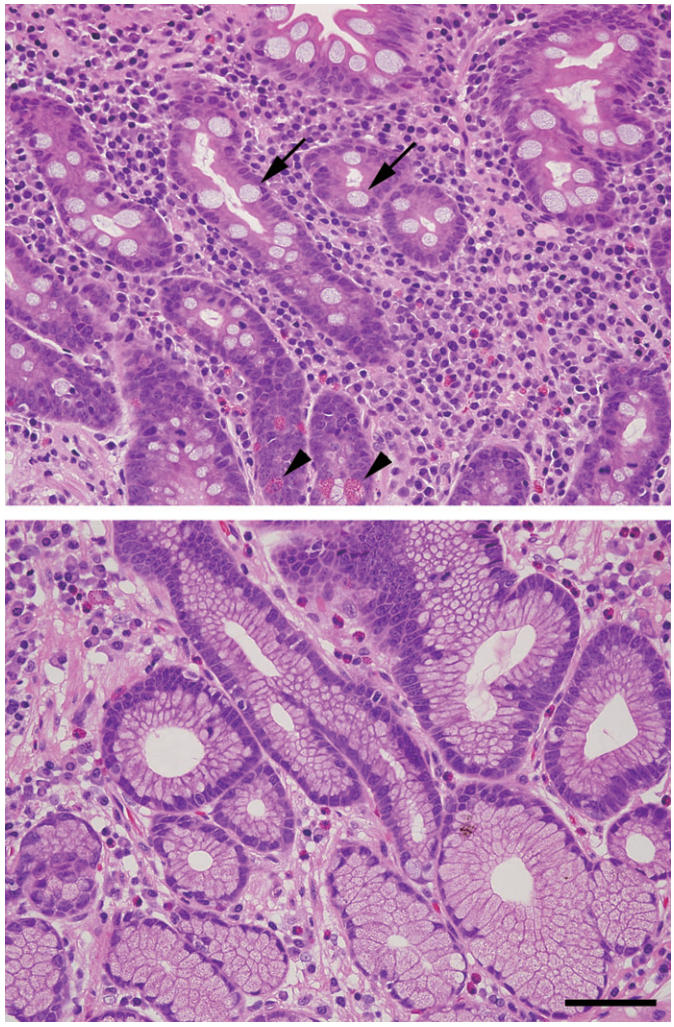

Fig. 1. Photomicrograph of gastric mucosa with (upper panel) or without (lower panel) intestinal metaplasia. (**A**) Complete-type intestinal metaplasia of gastric epithelial cells observed in chronically inflamed gastric mucosa. Gastric epithelial cells are replaced with absorptive cells, mucin-secreting goblet cells (shown by arrows), and Paneth cells having bright eosinophilic granules (shown by arrowheads). (**B**) Almost normal gastric epithelial cells in the pyloric gland area without intestinal metaplasia. These were stained by hematoxylin and eosin. Bar = 100 μm.

H. pylori reduces the risk of subsequent gastric adenocarcinoma irrespective of age, despite preexisting severe gastric atrophy or intestinal metaplasia. Population screening and treatment should now be pursued by governments in populations at very high risk and by WHO (Talley 2008).

In the course of inflammation to gastric cancer formation, chronic superficial gastritis leads to atrophic gastritis, intestinal metaplasia, dysplasia, and adenocarcinoma. In this sequence, an increased IL-1β secretion caused by inflammatory response facilitates the conversion from superficial gastritis to atrophic gastritis. Further mutation in RAS and loss of deleted in colorectal cancer (DCC) eventually results in gastric adenocarcinoma (Peek and Blaser 2002). As described below, this inflammation–carcinoma sequence is significantly facilitated by the CagA protein and a subtype of VacA. These proteins unique to *H. pylori* play important roles in gastric carcinoma formation.

MALT Lymphoma. Most lymphomas of the stomach are mucosa-associated lymphoid tissue (MALT) lymphoma, a low-grade B-cell lymphoma. This type of lymphoma arises in MALT, from which the name was derived. B cells that give rise to MALT lymphomas normally reside in the marginal zones of lymphoid follicles and are increased in response to various types of chronic inflammation, including chronic gastritis due to *H. pylori* infection (Isaacson and Wright 1984). Chronic infection with *H. pylori* leads to generation of *H. pylori*-reactive T cells, which, in turn, activate a polyclonal population of B cells by secreting soluble factors (Hussell et al. 1996). In time, a monoclonal but T-cell-dependent population of proliferating B cells emerges. Presumably, such monoclonal B-cell proliferation subsides when the antigenic stimulus for T cells is removed by antibiotic treatment. However, if untreated, genetic mutations accumulate in these proliferating B cells, and they eventually become T cell independent (Kumar et al. 2005).

Virulence factors

CagA. In the industrialized world, 60–70% of *H. pylori* strains express the cytotoxin-associated antigen A (CagA), a 120–145 kDa protein (Covacci et al. 1993). The cagA gene is localized at one end of the cag pathogenicity island (cag PAI), a 37 kb genomic fragment containing putative 31 genes (Tomb et al. 1997; Alm et al. 1999). Several of these are homologous to genes encoding the type IV secretion apparatus (Covacci et al. 1999). Upon direct contact of *H. pylori* with gastric epithelial cells, CagA is injected from the bacterium into the host cell via the type IV secretion system (Segal et al. 1999; Asahi et al. 2000; Odenbreit et al. 2000; Stein et al. 2000). After entering an epithelial cell, CagA is phosphorylated and binds to Src homology 2 domain-containing tyrosine phosphatase 2 (SHP-2), leading to a growth factor-like cellular response and cytokine production (Higashi et al. 2002). Deregulation of SHP-2 by CagA is an important mechanism by which CagA promotes gastric epithelial carcinogenesis (Ohnishi et al. 2008).

Recently, it was reported that CagA-positive strains induce the expression of several genes involved in glycan biosynthesis in gastric epithelial cells, in particular encoding β1,3-*N*-acetylglucosaminyltransferase 5 (β3GnT5) (Marcos et al. 2008), which is essential for the biosynthesis of Lewis antigens on glycolipids (Togayachi et al. 2001). This induction is dependent on CagA and CagE, most probably through the TNF/NF-κB pathway. The study identified a novel mechanism by which *H. pylori* modulates the biosynthesis of the sialic acid-binding adhesin (SabA) ligand in gastric epithelial cells, thereby increasing the epithelial attachment necessary to achieve successful colonization. Details of SabA are described below.

VacA. Vacuolating toxin (VacA) is a major virulence factor secreted by *H. pylori* and is a key component in the pathogenesis of gastric diseases (Cover and Blaser 1992). Approximately 50% of *H. pylori* strains express the VacA protein, and that expression is correlated with the expression of CagA. The most established activity of VacA is cellular vacuolation in mammalian cells (Catrenich and Chestnut 1992; Cover and Blaser 1992; Papini et al. 1993). Although the precise mechanism of VacA-induced vacuole formation is not fully understood, it involves binding and internalization of toxin. It has been proposed that vacuolation is a consequence of anion-selective channel formation in late endosomal compartments (Czajkowsky et al. 1999; Szabo et al. 1999; Tombola et al. 1999; Papini et al. 2001). In addition to its vacuole formation activity, VacA causes numerous cellular events, including depolarization of the membrane (Szabo et al. 1999; Schraw et al. 2002), apoptosis (Peek et al. 1999; Galmiche et al. 2000; Kuck et al. 2001; Willhite et al. 2003), interference with epithelial cell attachment (Fujikawa et al. 2003), and inhibition of T-lymphocyte activation (Gebert et al. 2003). These effects collectively contributed to the pathogenesis of *H. pylori*.

Glycoconjugates associated with *H. pylori*

Putative role of the Lewis blood group antigen in LPS of H. pylori

The cell wall of all Gram-negative bacteria is composed of two phospholipid bilayers with a peptidoglycan layer sandwiched between them. Lipopolysaccharide (LPS) is a structural component of the outer cell wall. LPS is composed of a long-chain fatty acid anchor called lipid A, a core sugar chain, and a variable carbohydrate chain designated O antigen, which is attached to the core sugar (McAdam and Sharpe 2005). Thus, the O antigen has the potential to exhibit enormous structural variability and is the domain determining the serological specificity of LPS (Moran and Prendergast 2001).

Clinical isolates of *H. pylori* produce the O antigen of a relatively constant chain length (Moran 1995). It is this region of *H. pylori* LPS that shares structural homology with Lewis blood group antigens in the gastric mucosa, predominantly Lewis X and Lewis Y antigens bearing type 2 blood group determinants. Serologically, 80–90% of *H. pylori* strains have been found to contain Lewis X and/or Lewis Y epitopes. Lewis blood group antigens are present in normal human gastric mucosa, and the expression of these antigens on *H. pylori* LPS has important biological implications. Molecular mimicry mediated by *H. pylori* LPS has been suggested to camouflage the bacterium and facilitate initial colonization (Edwards et al. 2000). However, immunogenicity of LPS is rather weak and a recent report showed that gastric H^+, K^+-ATPase is a major autoantigen in chronic *H. pylori* gastritis with body mucosa atrophy (Claeys et al. 1998).

Additionally, *H. pylori* Lewis antigens undergo phase variation: specifically, random, reversible high-frequency switching of phenotype contributes to virulence. The molecular mechanisms involved in phase variation are slipped-strand mispairing in poly-C tracts and translational frameshifting by ribosomal slippage (Wang et al. 2000). At least five glycosyltransferase genes are involved in generating phase variants: the genes encoding α3-fucosyltransferase (of which there are two similar but nonidentical copies), α2-fucosyltransferase, β3-galactosyltransferase, and β3-*N*-acetyl-D-glucosaminyltransferase (Appelmelk et al. 2000). Each of these genes can be either "on" or "off," and thus, in any *H. pylori* cell population, at least 32 different glycosyltransferase gene "on–off" combinations and potentially the same number of LPS phenotypes are present (Appelmelk et al. 2000). Thus, different *H. pylori* strains can potentially express different LPS Lewis phenotypes.

This antigenic mimicry may result in immune tolerance against antigens of the pathogen or in the induction of

autoantibodies that recognize gastric epithelial cells, which are frequently observed in patients with chronic active gastritis.

Adhesion of H. pylori to gastric epithelial cells

Attachment is a prerequisite for microbial colonization of epithelial surfaces and is mediated by molecules on the bacterial surface, adhesins, which recognize proteins or glycoconjugates on the surface of eukaryotic cells. The specificity of this interaction and the limited distribution of receptors often result in a restricted range of hosts and tissues utilized for colonization, a phenomenon known as tropism. Bacteria, which are unable to adhere to epithelia, tend to be rapidly removed by shedding from surface cells and the mucus layer.

H. pylori expresses adhesins that confer intimate adherence to the gastric epithelium where the bacteria can gain easy access to nutrients from host tissues (Aspholm-Hurtig et al. 2004). These adherence properties protect the bacteria from the extreme acidity of the gastric lumen and displacement from the stomach by forces such as those generated by peristalsis and gastric emptying (Ilver et al. 1998). Two carbohydrate structures in surface mucous cells serve as specific ligands for *H. pylori* adhesins: Lewis b, which binds to blood group antigen-binding adhesin (BabA), and sialyl dimeric Lewis X-bearing glycosphingolipid, which binds to sialic acid-binding adhesin (SabA). In addition, attachment of *H. pylori* to gastric epithelial cells can induce pedestal formation (Segal et al. 1996). Pedestal formation describes the creation of an upright support, constructed of host cell material, beneath an attached bacterium.

BabA. The best defined *H. pylori* adhesin–receptor interaction characterized to date is that between BabA, a member of a family of *H. pylori* outer membrane proteins, and Lewis b, H, and related ABO antigens (Ilver et al. 1998). These fucose-containing blood group antigens are found on red blood cells and in the gastrointestinal mucosa. Blood group O individuals suffer disproportionately from peptic ulcer disease (Ikehara et al. 2001), suggesting that bacterial adherence to H and Lewis b antigens influences severity of infection. The human population of South American Amerindians dominantly expresses the blood group O antigen. Interestingly, BabA from this population binds the blood group O antigen more efficiently than other blood group antigens (Aspholm-Hurtig et al. 2004). These findings suggest that *H. pylori* that binds to the blood group O antigen is preferentially increased during infection, and those bacteria gradually dominate among different *H. pylori*. BabA has two isoforms: babA1 and babA2. The product of the babA1 gene, in contrast to that encoded by the babA2 gene, cannot interact with Lewis b; thus it does not enhance *H. pylori* colonization of the surface epithelium (Ilver et al. 1998; Gerhard et al. 1999).

SabA. The sabA gene encodes a 651-amino-acid protein of 70 kDa and belongs to the large hop family of *H. pylori* outer membrane protein genes, which also includes the babA gene (Mahdavi et al. 2002). Sialyl dimeric Lewis X glycolipid is rarely expressed in normal gastric mucosa. However, the gastric mucosa infected by *H. pylori*, particularly CagA-positive strains, newly expresses this unique glycolipid in surface mucous cells partly facilitated by the increased expression of β3GnT5 (Marcos et al. 2008), which is essential for poly-*N*-acetyllactosamine synthesis in glycolipids (Togayachi et al. 2001). The adhesion mediated by SabA binding to sialyl dimeric Lewis X glycolipid thus contributes to persistent *H. pylori* infection established after the initial infection. Since βGnT5 expression levels are increased as inflammation progresses, *H. pylori* facilitates further infection by increasing ligands for the SabA adhesion molecule. Sialyl dimeric Lewis X is also expressed in leukocytes, but an "on–off" frameshift mutation of the SabA gene allows *H. pylori* to escape intimate contact with these inflammatory cells. Such adaptive mechanisms play an important role in the extraordinary chronicity of *H. pylori* infection in human gastric mucosa.

Induction of PNAd in gastric mucosa infected by *H. pylori*

In chronic inflammatory states, L-selectin and its ligands are implicated in lymphocyte recruitment in those diseases in which peripheral lymph node addressin (PNAd) is induced on high endothelial venule (HEV)-like vessels (von Andrian and Mackay 2000; Rosen 2004). Such HEV-like vessels have been observed in rheumatoid arthritis, lymphocytic thyroiditis, and inflammatory bowel diseases (Duijvestijn et al. 1987; Kabel et al. 1989; van Dinther-Janssen et al. 1990; Salmi et al. 1994; Suzawa et al. 2007). In these studies, the induction of PNAd is detected by the MECA-79 antibody (Streeter et al. 1988), which decorates PNAd on HEV-like vessels. MECA-79-positive HEVs in secondary lymphoid organs play a major role in lymphocyte homing (Rosen 2004). The MECA-79 epitope has been shown to be 6-sulfo *N*-acetyllactosamine attached to extended core 1 *O*-glycans, Galβ1→4(SO3→6)GlcNAcβ1→3Galβ1→3GalNAcα1→Ser/Thr (Yeh et al. 2001). Moreover, the MECA-79 antibody can also bind to its sialylated and fucosylated form that constitutes PNAd (Yeh et al. 2001). Structural studies also show that 6-sulfo sialyl Lewis X on core 2 branched *O*-glycans, sialic acidα2→3Galβ1→4[Fucα1→3(SO3→6)]GlcNAcβ1→6(Galβ1→3)GalNAcα1→Ser/Thr, is present as a major L-selectin ligand on HEVs (Hemmerich et al. 1995; Yeh et al. 2001). This structure is recognized by the NCC-ST-439 antibody (Kumamoto et al. 1998; Kobayashi et al. 2004).

In *H. pylori*-infected gastric mucosa, HEV-like vessels can be stained by MECA-79 and NCC-ST-439 antibodies. This indicates that PNAd in gastric mucosa formed by *H. pylori* infection is similar, if not identical, to PNAd present in secondary lymphoid organs, which is formed under normal condition (Yeh et al. 2001). The number of HEV-like vessels, as detected by MECA-79 and HECA-452 antibodies, correlates positive progression of chronic inflammation in *H. pylori*-infected gastric mucosa. Surprisingly, the gastric mucosa no longer displayed HEV-like vessels after the eradication of *H. pylori* (Kobayashi et al. 2004) (Figure 2). These findings indicate that the infection by and the presence of *H. pylori* is a primary cause to induce and maintain PNAd on HEV-like vessels in infected gastric mucosa, thereby facilitating inflammatory response. Recently, it was reported that PNAd presented by *N*-glycans is almost as an efficient ligand as that on *O*-glycans (Mitoma et al. 2007). These findings suggest that PNAd carried by *N*-glycans on HEV-like vessels also functions in facilitating inflammatory response in *H. pylori*-infected gastric mucosa.

It is tempting to speculate that bacterial components such as LPS acting through Toll-like receptor-dependent pathways in the gastric epithelium stimulate the release of cytokines, i.e.,

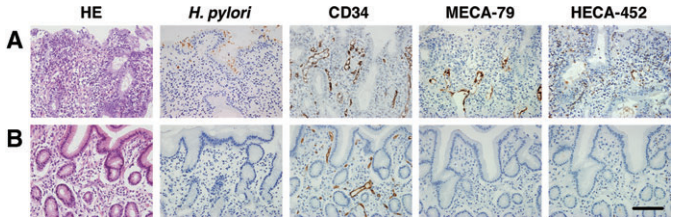

Fig. 2. Disappearance of HEV-like vessels in the gastric mucosa after the eradication of *H. pylori*. Gastric mucosa infected with *H. pylori* was examined before and 2 months after treatment to eradicate *H. pylori*. (**A**) Before treatment, HEV-like vessels detected by MECA-79 and HECA-452 antibodies were abundant, and large numbers of mononuclear cells (lymphocytes) were present around these vessels. (**B**) After the eradication of *H. pylori*, HEV-like vessels were no longer present and very few mononuclear cells were present. CD34 was used for a marker of vascular endothelial cells. HE, hematoxylin and eosin, Bar = 100 μm. Adapted with permission from Kobayashi et al. (2004).

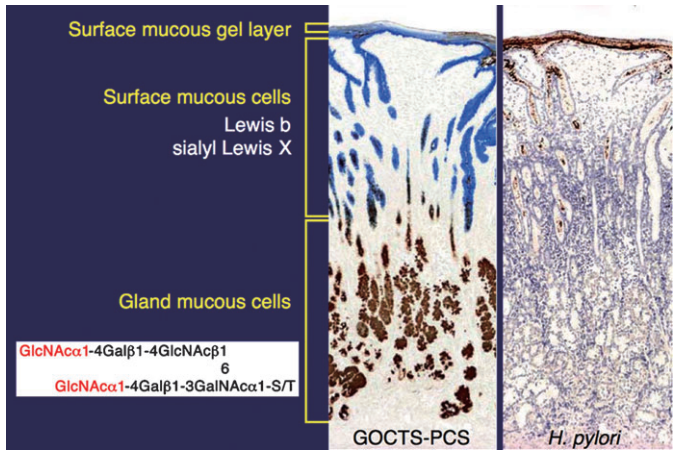

Fig. 3. Two distinct mucins present in gastric mucosa. Surface mucous cell-derived mucins containing Lewis b and sialyl Lewis X (in blue), and gland mucous cell-derived mucins containing α1.4-GlcNAc-capped *O*-glycans (in brown) can be distinguished by galactose oxidase-cold thionin Schiff-paradoxical concanavalin A staining (GOCTS-PCS) (left panel). *H. pylori* in brown in the right panel is almost exclusively present in the surface mucous-cell derived mucin.

lymphotoxin α (Pablos et al. 2005). This effect might in turn modulate gene expression in postcapillary venules in a way that could cause their biochemical, functional, and morphological transformation by upregulating chemokines, such as CCL19 and CCL21 that act on CCR7 receptors (Drayton et al. 2003).

Roles of gastric mucin in *H. pylori* infection

Gastric mucins are classified into two types based upon their histochemical properties (Ota et al. 1991): one is a surface mucous cell-derived mucin displayed on the MUC5AC core protein (Reis et al. 1999) and the other is a mucin displayed on the MUC6 core protein secreted by gland mucous cells, including cardiac gland cells, mucous neck cells, and pyloric gland cells (Reis et al. 2000). These two mucins form the surface mucus gel layer (SMGL), which shows an alternately laminated array. The thicker layer mostly consists of MUC5AC and MUC6 (Ho et al. 2004). However, the most recent report showed that MUC6 is negligible in SMGL (Phillipson et al. 2008), in supporting the conclusion that SMGL mostly consists of MUC5AC.

H. pylori is exclusively associated with surface mucous cell-derived mucins and rarely colonizes deeper portions (Figure 3). *H. pylori* density correlates with the Lewis b antigen that is presented on MUC5AC glycoproteins (Van de Bovenkamp et al. 2003). As MUC5AC constitutes a major component of surface mucosa mucins (Nordman et al. 2002), *H. pylori* mostly resides in surface mucosa.

In contrast to MUC5AC, MUC6 is expressed in deeper portion of the mucosa, and its mucin-type *O*-glycans are capped by α1,4-linked *N*-acetylglucosamine (α1,4-GlcNAc) (Nakayama et al. 1999). MUC6 rather excludes the colonization of *H. pylori*.

These findings suggest that mucin-type *O*-glycans on MUC6 may inhibit *H. pylori* growth. Indeed, *H. pylori* growth was inhibited by the presence of mucin proteins such as CD43, which express α1,4-GlcNAc-capped *O*-glycans with the concomitant decrease in cholesterol α-glucoside in *H. pylori* cell wall (Kawakubo et al. 2004). Cholesterol α-glucoside and its derivatives, synthesized by cholesterol α-glucosyltransferase, constitute 25% of *H. pylori* cell wall lipids (Hirai et al. 1995; Haque et al. 1996). Morphological abnormality of *H. pylori* after incubation with those recombinant mucin proteins expressing α1,4-GlcNAc-capped *O*-glycans may be due to the decrease in cholesterol α-glucoside.

Significantly, the decrease in cholesterol α-glucoside either by inactivation of the enzyme or increase in cholesterol resulted in higher susceptibility to macrophage and increased response by T lymphocytes (Wunder et al. 2006). Similarly, synthetic oligosaccharides containing the α1,4-GlcNAc-capping structure inhibit in vitro activity of cholesterol α-glucosyltransferase from *H. pylori* (Lee et al. 2006) and *H. pylori* growth (Lee et al. 2008) (Figure 4). In parallel to these findings, porcine mucin inhibits *H. pylori* growth most likely due to the binding of *H. pylori* to the mucin, which expresses blood group antigens (Gustafsson et al. 2006). It is also possible that this inhibition shares a similar or the same mechanism where mucins containing the α1,4-GlcNAc-capping structure inhibit *H. pylori* growth. The mechanism how mucins expressing α1,4-GlcNAc-capping structures can inhibit *H. pylori* growth needs to be elucidated. In a separate study, *H. pylori* infection was treated with sialic acidα2→3Galβ1→4Glc (Miller-Podraza et al. 2005) with a modest success (Mysore et al. 1999). As this oligosaccharide inhibits *H. pylori* adhesion mostly in gastritis patients, Lewis b-containing glycans may be useful in preventing BabA-mediated adhesion that also functions in early stages of *H. pylori* infection.

These findings would point to a possibility that α1,4-GlcNAc-capped *O*-glycans may be useful for treatment of *H. pylori* infection. This possibility becomes more reasonable if oligosaccharides with multiple α1,4-GlcNAc residues are synthesized. However, the effective concentration of inhibitory oligosaccharide containing a monovalent α1,4-GlcNAc residue is very high (~0.5 mM) (Lee et al. 2008), and it is likely that the oligosaccharide approach may not be practical. On the other hand, the inhibition of cholesterol α-glucosyltransferase should lead to increased susceptibility to immune response by innate immunity and T-cell response. Identifying the cholesterol α-glucosyltransferase inhibitor of low molecular weight is thus an important task yet to be explored.

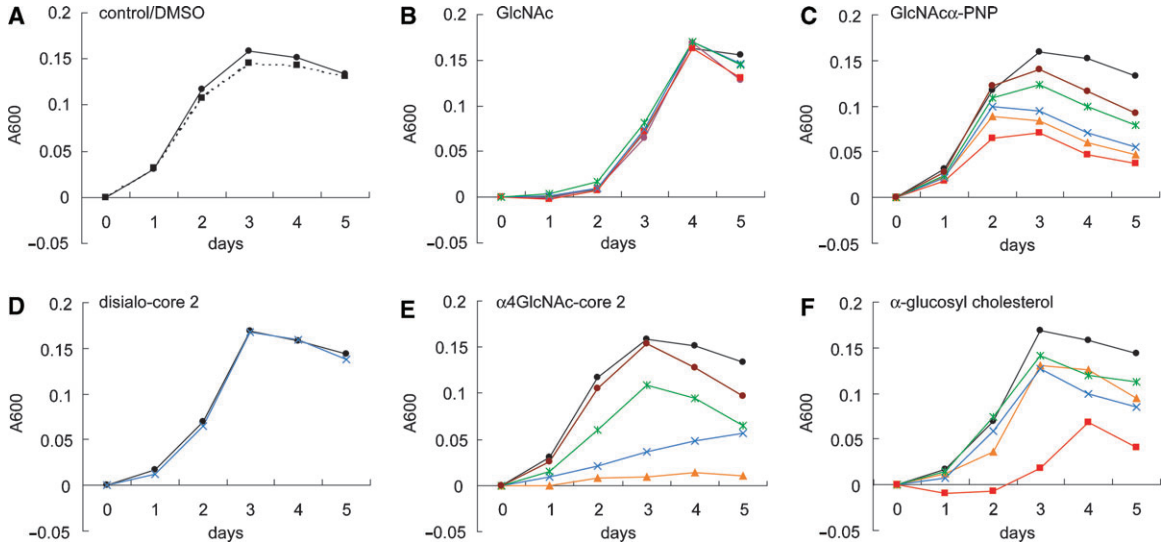

Fig. 4. Inhibition of *H. pylori* growth by synthetic oligosaccharides and monosaccharides. *H. pylori* was cultured for 5 days in Mueller–Hinton broth supplemented with 5.5% horse serum containing various amounts of synthetic oligosaccharides and monosaccharides. Bacterial growth was measured at O.D. 600 nm, and the absorbance for control experiments at time 0 was subtracted from absorbance at later time points. Oligosaccharide and monosaccharide concentrations are 1 mM (red), 0.75 mM (orange), 0.5 mM (blue), 0.25 mM (green), 0.125 mM (brown), and control (closed circle). Two mM GlcNAc were also added in B (magenta). Oligosaccharides and monosaccharides were initially dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration in the culture medium was 1%. The growth curve in the absence of DMSO is shown as a dotted line (**A**) Adapted from Lee et al. (2008).

Conclusion

In this review, we have shown that a battery of *O*-glycans such as 6-sulfo sialyl Lewis X and α1,4-GlcNAc-capped *O*-glycans expressed in the HEV-like vessels and gland mucous cells, respectively, plays pivotal roles on pathogenesis of chronic active gastritis and on protection of the gastric mucosa from *H. pylori*, respectively (Figure 5). Moreover, Lewis b and sialyl dimeric Lewis X on mucus cells serve as counterreceptors for adhesins coded by *BabA* and *SabA*, respectively. In the healthy gastric mucus layer, BabA and acid charge affect binding to mucin, while in gastritis patients, BabA/Lewis b-dependent binding to

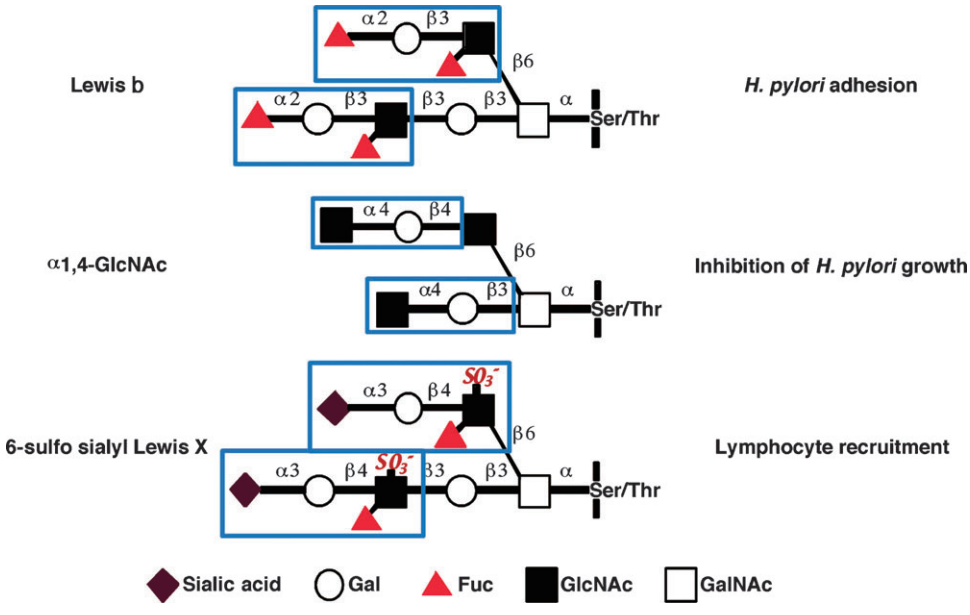

Fig. 5. Carbohydrates critical for *H. pylori* infection and pathogenesis. Distinct sets of carbohydrates on gastric mucosa play critical roles in *H. pylori* adhesion, inhibition of *H. pylori* growth, and recruitment of lymphocytes and facilitation of inflammatory response following *H. pylori* infection. BabA adhesin on *H. pylori* binds to Lewis b blood group antigen, thus facilitating *H. pylori* colonization (Ilver et al. 1998). This colonization is counteracted by α1,4-GlcNAc-capped *O*-glycans present in the deeper portion of gastric mucosa (Kawakubo et al. 2004). Once *H. pylori* infects the stomach a series of inflammatory responses is initiated, and this response is facilitated by the de novo expression of 6-sulfo sialyl Lewis X on HEV-like vessels that recruit lymphocytes to inflammatory sites (Kobayashi et al. 2004).

MUC5AC remains, but SabA and low pH binding increases (Mahdavi et al. 2002; Linden et al. 2008). After *H. pylori* established its infection, the inflammatory response to *H. pylori* infection leads to the formation of PNAd in HEV-like vessels, which facilitate lymphocyte recruitment to inflammatory sites, enhancing inflammatory response. These findings demonstrate that distinct sets of carbohydrates play critical roles in adhesion of *H. pylori* to epithelial cells (Lewis b, blood group O antigens, sialyl dimeric Lewis X), attenuation of *H. pylori* colonization (α1,4-GlcNAc-capped *O*-glycans), and facilitation of inflammatory response following *H. pylori* infection (6-sulfo sialyl Lewis X). These discoveries allow us to not only understand the pathogenesis of *H. pylori*-associated diseases but also to develop new therapy or prevention toward *H. pylori* infection by inhibiting these enzymes that are important for infection and colonization of *H. pylori*.

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Conflict of interest statement

None declared.

Abbreviations

β3GnT5, β1,3-*N*-acetylglucosaminyltransferase 5; BabA, blood group antigen-binding adhesin; CagA, cytotoxin-associated antigen A; DCC, deleted in colorectal cancer; HEV, high endothelial venule; LPS, lipopolysaccharide; MALT, mucosa-associated lymphoid tissue; PNAd, peripheral lymph node addressin; SabA, sialic acid-binding adhesin; SHP-2, Src homology 2 domain-containing tyrosine phosphatase 2; SMGL, surface mucous gel layer; VacA, vacuolating toxin.

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