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Abstract

Helicobacter pylori may cause gastritis, gastric/duodenal ulcer and gastric cancer. During infection, most H. pylori are found in the gastric mucus layer, but some are attached to, or have penetrated, epithelial cells. The aim of this study is to characterize the binding of H. pylori to gastric mucins and to investigate host changes that occur after inoculation. Results: H. pylori binds to mucins by at least 3 different mechanisms: 1) to host Leb and related structures via the BabA adhesin, 2) to host sialyl-Lex via the SabA adhesin and 3) to sialylated host structures at low pH. H. pylori strains expressing the BabA adhesin bind to the human MUC5AC mucin via Leb and to a gastric low-molecular-mass mucin-like molecule (possibly MUC1) via the H-type-1 structure. Leb-positive MUC5AC glycoforms differed in their receptor properties for different H. pylori strains. At pH 3, Leb binding was abolished, although all strains bound to a proteoglycan containing chondroitin sulfate/dermatan sulfate chains, to a component behaving as a monomeric mucin of higher charge and larger size than the subunits of MUC5AC/MUC6, and to a highly charged MUC5AC glycoform. Rhesus monkey and human gastric mucins are similar with respect to tissue localization, size, density, glycoforms, terminal carbohydrate substitution and H. pylori binding. After H. pylori inoculation, 8 of 10 monkeys developed persistent infection accompanied by gastritis. muc5AC and muc6 localized to the mucous cells of the surface epithelium and to the glands respectively, and no muc2 or sulfo-mucins were detected during the 10-month period investigated. A transient increase in sialylated Lewis antigens occurred as early as one week after inoculation. Furthermore, Lea and/or Leb expression briefly decreased in 5/7 Leb-positive animals, but later tended to increase, and in Lea/Leb negative animals Lea and/or Leb increased to a variable extent. H. pylori adherence in vitro reflects these glycosylation changes in that an increased binding with a sialyl-Lex binding strain, and an initial decrease in adherence by a Leb-binding one were observed, suggesting that the SabA and BabA adhesins play complementary roles during infection. Conclusions: H. pylori binding to mucins at neutral pH is strain, blood-group and glycoform dependent whereas binding at acidic pH seems to be a common feature for all strains. The host responds rapidly to bacterial challenge by changing the expression of carbohydrate structures used by the microbe for adhesion.

Key words: H. pylori, mucin, mucin glycoform, MUC5AC, host-pathogen interaction, gastric protection

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LIST OF PAPERS

This thesis is based on the following papers referred to in the text by their Roman numerals.

I. Sara Lindén, Henrik Nordman, Jan Hedenbro, Marina Hurtig, Thomas Borén, and Ingemar Carlstedt. Strain- and blood-group dependent binding of *Helicobacter pylori* to human gastric MUC5AC glycoforms. Gastroenterology 2002;123:1923-1930. Reprinted from with permission from the American Gastroenterological Association.

II. Sara Lindén, Jafar Mahadavi, Jan Hedenbro, Thomas Borén, and Ingemar Carlstedt. Effects of pH on *Helicobacter pylori* binding to human gastric mucins – identification of binding to non-MUC5AC mucins. Manuscript.

III. Sara Lindén, Thomas Borén, André Dubois and Ingemar Carlstedt. Rhesus monkey gastric mucins: Oligomeric structure, glycoforms and *Helicobacter pylori* binding. In press, Biochemical Journal 2004.

IV. Sara Lindén, Jafar Mahdavi, Cara Olsen, Thomas Borén, Ingemar Carlstedt and André Dubois. Effects of *Helicobacter pylori* inoculation on host glycosylation and *H. pylori* adhesion sites in rhesus monkey. Manuscript.

ABBREVIATIONS

AlpA	Adherence-associated lipoprotein A
AlpB	Adherence-associated lipoprotein B
BabA	Blood-group binding adhesin
CF	Cystic fibrosis
DU	Duodenal ulcer
ECM	Extracellular matrix
EGF	Epidermal growth factor
Fuc	Fucose
Gal	Galactose
GalNAc	*N*-acetylgalactosamine
GlcNAc	*N*-acetylglucosamine
GI	Gastrointestinal
IL	Interleukin
Le	Lewis
LPS	Lipopolysaccharides
MAL	*Maakia amurensis* II
NAP	Neutrophil activating protein
NLBH	Neuraminyl-Lactose-Binding Adhesin
PAI	Pathogenicity island
PNA	Peanut agglutinin
PUD	Peptic ulcer disease
SabA	Sialic acid binding adhesin
TNF-α	Tumor necrosis factor alpha
TR	Tandem repeat
VNTR	Variable number of tandem repeats
vWF	Von Willebrand factor

INTRODUCTION

The average person carries in the order of 10^{14} bacteria, which live in harmony with our mere 10^{13} host cells. The majority of these microbes protects the host from pathogens and some even provide nutrients. However, the human body also encounters potentially pathogenic bacteria every day although establishment of infections after such contacts is rare relative to the frequency of exposure. The skin and mucosal surfaces, which comprise the barrier between the body and the external milieu, are the first lines of defense, and colonization of these surfaces is normally the first step in bacterial disease. In adult humans, the area of the skin is approximately 2 m^2 whereas the mucosal surfaces together comprise about 400 m^2. As a physical barrier, the skin is reinforced by dry, slightly acidic conditions, sloughing of cells and a resident microflora. The non-keratinized mucosal surfaces on the other hand, have additional functions such as secretion of digestive enzymes and absorption of nutrients that require the barrier to be 'semi-permeable' with ensuing demands on their design. In addition to sloughing of cells and a resident microflora, the mucosal surfaces are protected by a mucus layer. This gel-like layer is formed by highly glycosylated proteins referred to as mucins and is efficient in trapping microorganisms. The mucus gel is constantly shed on the luminal side and replenished by secretion from the underlying epithelium, resulting in a continuous 'washing' of the mucosal surface. In addition, the mucus gel contains protective proteins that defend against pathogens, and in the gastrointestinal (GI) tract bacteria are killed by acid and bile salts. The underlying cells relay signals to activate the immune system and mobilization of tissue and blood defense mechanisms is an important aspect of host defense. However, such mechanisms may be accompanied by tissue damage, and infectious diseases are often aggravated by an excessive host response to the invading pathogen. Invading pathogens also have weapons (virulence factors) to fight the host with, for example toxins and mucin-degrading enzymes which may disrupt the mucus layer.[1] However, due to the efficient host defense mechanisms, most interactions are benign or short, and only when the microbe avoids early killing by the host can a persistent infection be established. Within the human population there is considerable variation in the ability to resist infections and one individual may effectively ward off a certain pathogen while being susceptible to attack from another.

This thesis describes some aspects of host-pathogen interactions at the interface between the inside and outside of the body. The interaction of the ulcerogenic and carcinogenic bacterium *Helicobacter pylori* with the gastric mucosa is used as a model and the work is focused on mucins and mucin glycosylation.

BACKGROUND

The mucosal surface – a barrier against the outside world

Most pathogens cause disease by disrupting and/or penetrating mucosal surfaces, and in order to be successful they have to circumvent the host defense. The first barrier the pathogen encounters is the highly hydrated mucus gel that covers the mucosal surface and protects the epithelial cells against chemical, enzymatic, microbial and mechanical insult. The mucus gel is formed by high-molecular-mass oligomeric glycoproteins (mucins) and protection is reinforced by a number of 'defense factors' (Table 1) trapped in the gel matrix. The thickness of the mucus layer is highly variable depending on, for example, tissue location. In addition, the data reported vary with the method used for the measurements. In a study performed on the rat GI tract *in vivo*, the mucus gel was found to consist of a firmly adherent layer with a thickness of 15-154 μm and a loosely adherent one of 108-714 μm.[2] The functional significance of the two layers has not been established, but their presence may be explained by differences in mucin concentration.[2] Underneath the mucus layer, the cells present a dense forest of highly diverse glycoproteins and glycolipids, which form the glycocalyx (Figure 1). Again, the thickness is highly variable; for example the glycocalyx of cat and human intestinal microvilli tips is 0.1-0.5μm thick whereas that of the lateral microvilli surface is 30-60 nm. [3-5] In the electron microscope, the glycocalyx appears as filaments attached to the plasma membrane. The oligosaccharide moieties of the molecules forming the glycocalyx and the mucus layer are highly diverse and the average turnover time of the human jejunum glycocalyx is 6-12 hours.[6] Consequently, the mucosal surfaces presented to the outside world are constantly renewed and could potentially be adjusted to changes in the environment, *e.g.* microbial attacks. Alteration in glycosylation has been proposed to influence cell adhesion, receptor activation, cell differentiation, and tissue morphogenesis.

Protein	Function
Lysozyme	Digests bacterial peptidoglycans, *i.e.* breaks down the bacterial cell wall[7]
Lactoferrin	Prevents bacterial growth by binding iron
Lactoperoxidase	Kills bacteria by generating superoxide radicals
Defensins	Introduce ion-permeable channels in the bacterial cell membrane[7]
Trefoil factors	Involved in wound healing
Secretory IgA	*e.g.* prevents bacterial attachment to mucosal cells[7]

Table 1. Examples of 'defense proteins' present in mucus.

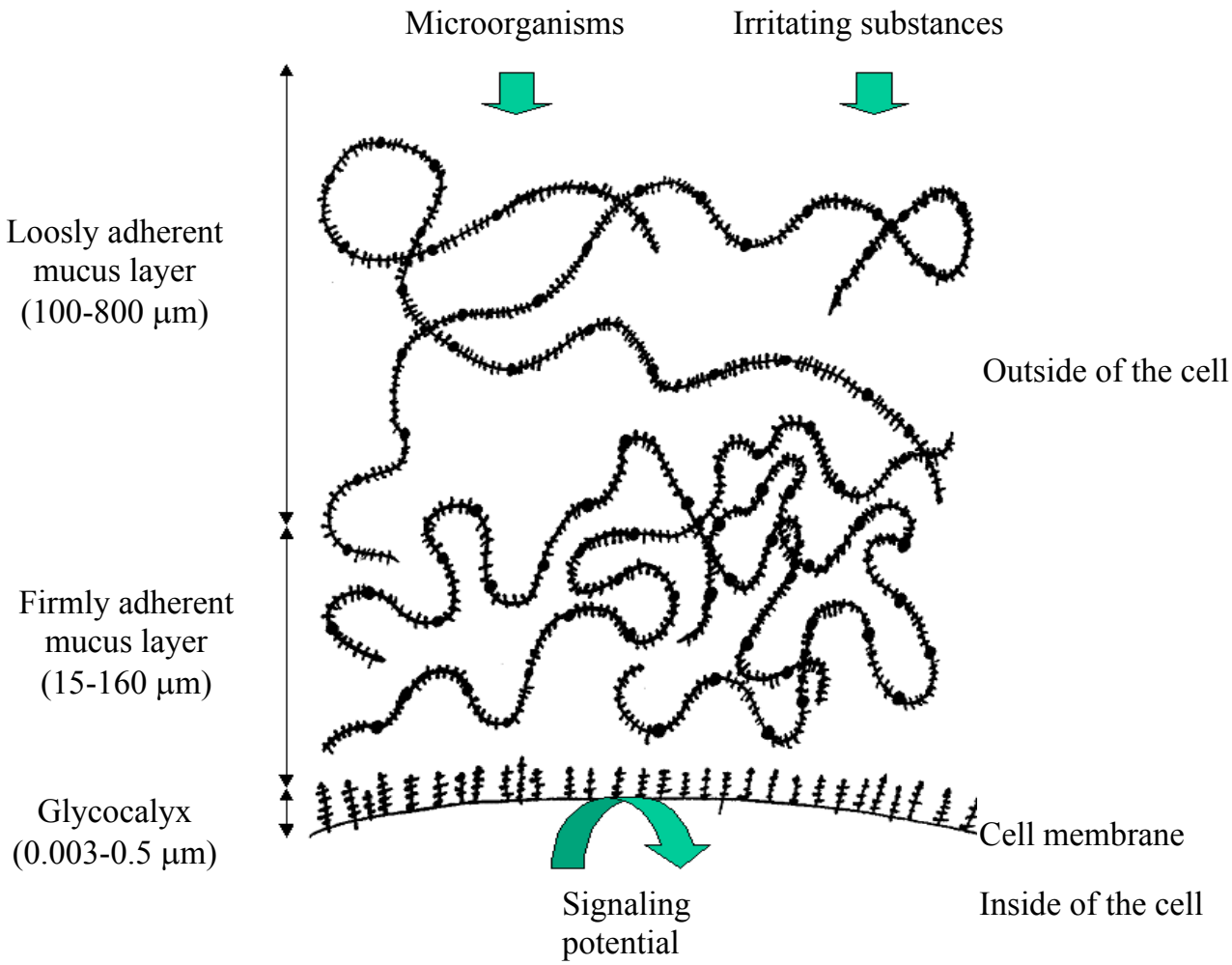


Figure 1. Pictorial representation of the mucosal barrier consisting of the mucus layer and the glycocalyx. The mucins comprising the mucus layer are composed of subunits joined by disulphide bonds. The threadlike oligomers have regions with a high oligosaccharide density giving these domains a 'bottle–brush' appearance. The oligosaccharide chains are depicted as short lines perpendicular to the mucin apoprotein and the link between the subunits as •.

The stomach

The stomach disintegrates food mechanically and chemically. The acidic gastric juice and proteolytic enzymes kill many of the bacteria that are swallowed and individuals with achlorhydria are more susceptible to intestinal infections than those with normal gastric pH.[8] Acid secretion is induced by a variety of stimuli and governed by the endocrine cells of the stomach. Gastrin is released from the G cells of the antral mucosa and travels through the blood stream to the corpus where the entero-chromaffin like cells are stimulated to secrete histamine which, in turn, stimulates the parietal cells to secrete acid.[9] In contrast, somatostatin, prostaglandins, interleukin (IL)-1β and epidermal growth factor (EGF) inhibit acid secretion.[9] The viscous mucus layer (average thickness of the human firmly adherent layer 144 μm[10]) acts as a barrier for large molecules thus protecting the gastric mucosa against *e.g.* pepsin. In addition, mucus acts as an 'unstirred layer' in which bicarbonate ions secreted by the surface epithelium counteract protons diffusing from the lumen into the gel, causing

the pH in the mucus layer to range from acidic in the lumen to neutral at the cell surface (Figure 2).[11] Bicarbonate ion secretion is stimulated by the presence of acid in the gastric lumen and transport across the apical epithelial membrane probably occurs by chloride ion/bicarbonate ion exchange.[11]

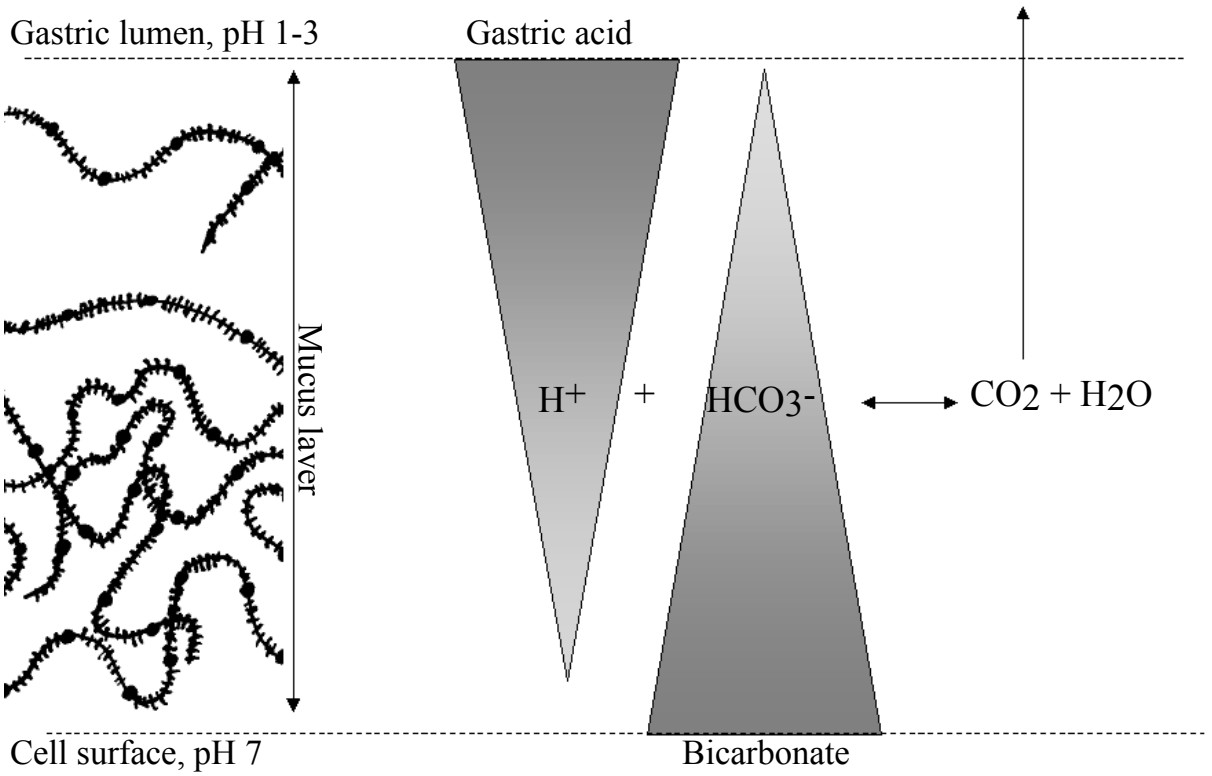


Figure 2. The pH gradient in the gastric mucus layer. The pH gradient ranging from acidic in the lumen to neutral at the cell surface is formed by opposing diffusion of luminal acid and bicarbonate secreted by the surface epithelial cells.[11]

The gastric mucosa

In the stomach, the surface epithelium is connected via the foveolae and neck region to the deeper gastric glands (Figure 3). The surface/foveolar epithelium is formed by a single layer of tall columnar mucin-producing cells that have a basal nucleus below an apical cup of mucin. These cells have a turnover rate of 3-6 days.[12] Undifferentiated stem cells are located in the neck region (Figure 3) and migrate outwards along the foveolar axis differentiating into mucous cells, and inwards differentiating into mucous, parietal, chief and endocrine cells (Figure 3).[12] Outward migration is fast and the cells reach the lumen in about 2-3 days, whereas inward migration is slower; the bottom of antral/pyloric glands is reached in about 14 days and the bottom of corpus/fundic glands in about 200 days. The lamina propria contains inflammatory cells (plasma cells, lymphocytes and eosinophils), which are more numerous toward the mucosal surface and the antrum.[13] Lymphoid aggregates are found dispersed along the muscularis mucosa, especially in *H. pylori* infection.[13]

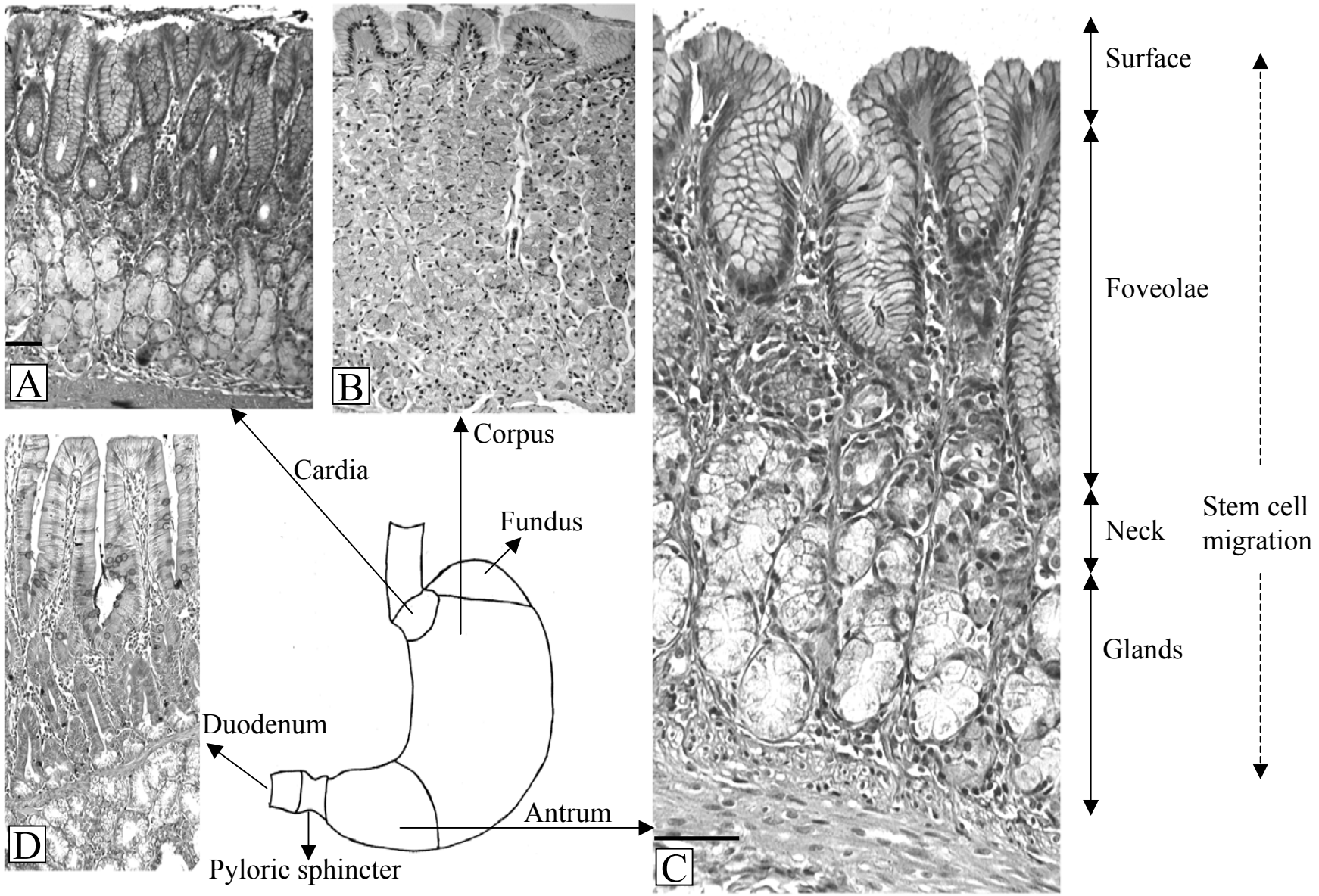


Figure 3. The regions of the stomach. Cardia. (A), corpus/fundus (B), antrum (C) are histologically different. In the gastric mucosa, the surface/foveolar epithelium is covered by a single layer of tall columnar mucin-producing cells whereas in the duodenum (D) mucin-producing goblet cells are interspersed among the enterocytes. In the stomach, undifferentiated stem cells located in the neck region migrate outwards or inwards specializing into mucous, parietal, chief and endocrine cells. The bars in panels a and c indicate 50 μm.

Histologically, the stomach is divided into three different regions – cardia, corpus/fundus and antrum/pylorus (Figure 3). The cardia comprises the proximal 0.5-2 cm of the stomach,[9] and the gastric foveolae occupy approximately half the mucosal thickness in this region (Figure 3A). The glandular compartment is composed of coiled glands lined by cuboidal mucus-producing cells and scattered pyramidal shaped parietal cells. In corpus and fundus, the gastric pits are shallow and occupy 1/4th or less of the mucosal thickness. Here the glands are unbranched (Figure 3B). Most cells in the upper part of the glands are acid-secreting parietal cells, whereas the lower half of the glands is dominated by cuboidal chief (peptic) cells that secrete digestive enzymes (pepsinogen and lipase). Most of the endocrine cells are found in the lower third of the glands. The antrum/pylorus occupies the distal 1/4th of the stomach. The tissue architecture of antrum/pylorus is similar to that of the cardia with foveolae occupying approximately half the mucosal thickness and the glandular compartment being composed of coiled glands lined by cuboidal mucous cells (Figure 3C). Sparse parietal cells and endocrine cells are located in the connecting neck region, and the endocrine cells are dominated by gastrin-producing (G) cells.[9]

The gastric content is emptied into the duodenum. The normal duodenal mucosa is lined by a columnar epithelium with absorptive cells and goblet cells, and have Brunner's glands/cells in the submucosa (figure 3D).[14] In gastric metaplasia of the duodenum, this region displays gastric characteristics necessary for *H. pylori* colonization.

Mucins – an integral part of the barrier

The mucus polymer matrix is formed by large (in the order of 10^7 Da) secreted mucins, which confer viscoelastic properties. In addition, there are membrane-associated mucins, which are smaller than the gel-forming ones, but still larger than most other membrane-bound glycoproteins. Each mucin gene contains unique tandem repeat (TR) motifs coding for regions with a high density of serine, threonine and proline. The TR varies in length between the mucins, and there is a genetic polymorphism in the number of repeats referred to as Variable Number of Tandem Repeats (VNTR) polymorphism. The VNTR polymorphisms cause mucin size to differ between individuals (Figure 4). The serine and threonine residues can be *O*-glycosylated and more than 50% (often 70-80%) of the mucin molecular mass is due to carbohydrate. Each mucin carries in the order of 100 different oligosaccharide structures.[15] These carbohydrate chains are often clustered into highly glycosylated domains, giving the mucin a 'bottle-brush' appearance. To date, at least 14 human mucins have been described, and the expression profile of mucins varies between tissues (table 2).

The secreted gel-forming mucins are oligomeric structures formed by subunits linked by disulfide bridges. Monomeric mucins can also be secreted as, for example, salivary MUC7. The mucins are produced by cells in the epithelial surface and/or by glands located in the submucosal connective tissues and secretion occurs via both constitutive and a regulated pathways.[16] The constitutive pathway continuously secretes a small amount of mucin to maintain the mucus layer, whereas the regulated pathway affords a massive discharge as a response to environmental and/or (patho)physiological stimuli.[16] Stimulated mucin release occurs rapidly and is accompanied by a hundredfold or so expansion of the secretory granules.[16]

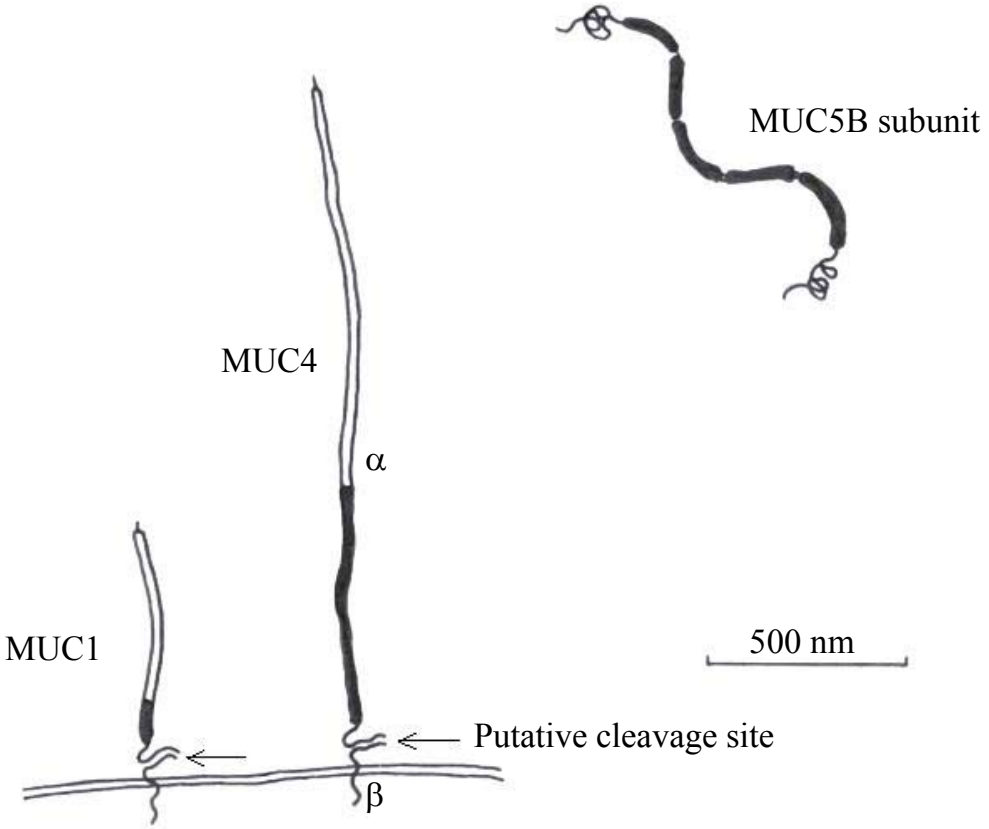


Figure 4. Schematic drawing of MUC1, MUC4 and a MUC5B subunit drawn to scale relative to each other.[17] The length of the glycosylated domains differ between alleles and here the filled part indicates the shortest allele whereas the filled together with the open part represents the longest one. The lengths of the glycosylated domains are estimated with the assumption that the length/amino acid is 0.25nm in this part of the molecule.[17] Reproduced by the permission of the authors and the Novartis Foundation.

The membrane-associated mucins provide a barrier that limits access of other cells and large molecules to the cell surface. These glycoproteins have a hydrophobic membrane-spanning domain, a C-terminal cytoplasmic 'tail' with putative sites for serine and tyrosine phosphorylation, SEA modules located in the extracellular region (possibly for signaling through dimerization[18]) and EGF–like domains.[19-23] The membrane-associated mucins can occur in secreted, non-membrane bound forms as a result of alternative splicing or proteolysis.[24, 25] MUC1 is an extensively studied membrane-associated mucin, and in addition to the membrane-bound form, secreted variants of this mucin have been reported.[24, 26] The gene products of the splice variants have been described to have different signal sequences, suggesting that they may be differently processed.[27] MUC1 towers 200-500 nm (depending on the number of tandem repeats) above the plasma membrane,[28] and is thus 'taller' than most other glycoproteins at the cell surface. The full length cDNA of MUC1 encodes an amino terminal domain consisting of a signal peptide and degenerative repeats, a large VNTR (Figure 4), and a carboxy-terminus consisting of degenerate repeats, a mucin-like unique sequence, a hydrophobic membrane-spanning stretch and a highly conserved cytoplasmic domain.[26] MUC1 is cleaved in the endoplasmic reticulum somewhere between 53-71 amino acids upstream of the trans-membrane region (in the SEA module), and the cleavage products remain associated as a heterodimer through non-covalent interactions.[29] MUC1 associated with the cell-surface is constantly internalized (0.9% of the surface fraction/min) and recycled.[30] Internalization occurs by clathrin-mediated endocytosis, and alterations in *O*-glycan structure stimulate endocytosis and intracellular accumulation.[31] During recycling, sialic acid is added to the premature form of MUC1.[30] Complete sialylation requires several rounds of recycling, one cycle taking approximately 2.5h.[30] Pulse-chase experiments indicate that the half-life of MUC1 in the plasma membrane is 16-24 hours[30, 32] suggesting that the average MUC1 molecule recycles up to 10 times before release.[30] The cytoplasmic tail appears to interact with the cytoskeleton and secondary signaling molecules whereas the extracellular domain interacts with extracellular matrix components and other cells.[33, 34] In addition, the MUC1/Y transmembrane splice variant lacking the TR region may interact with the secreted MUC1/SEC causing phosphorylation of the cytoplasmic tail and alterations in cell morphology, which suggests that the MUC1 gene may generate both a ligand and its receptor.[35] muc1 null mice display chronic infection and inflammation of the reproductive tract, reducing fertility rates, although only normal endogenous bacteria were isolated suggesting that these species become opportunistic with loss of muc1.[36] In addition, muc1 null mice have a high frequency of eye inflammation/infection involving *Corynebacteria, Staphylococci* and *Streptococci*.[37] In humans, there is an increased frequency of homozygotes for the short allelic form of MUC1 among humans with *H. pylori* gastritis.[38] Consequently, MUC1 is likely to play an important role in mucosal defense.

Mucin	Distribution	Mucin type	References
MUC1	Stomach, breast, gallbladder, cervix, pancreas, airways, duodenum, colon, kidney, B cells, T cells, dendritic cells	Membrane-bound and secreted	24, 33, 39, 40
MUC2	Small intestine, colon	Secreted, oligomeric	41, 42
MUC3A/ MUC3B	Small intestine, colon, gall bladder, duodenum	Membrane-bound and secreted	43, 44 45
MUC4	Trachea, colon, stomach, cervix, lung	Membrane-bound and secreted	19, 46
MUC5AC	Airways, stomach, endocervix	Secreted, oligomeric	47
MUC5B	Airways, salivary glands, cervix, gallbladder	Secreted, oligomeric	17, 45 47
MUC6	Stomach, duodenum, gallbladder, pancreas, seminal fluid, cervix	Secreted, oligomeric	48 47 45
MUC7	Salivary glands	Secreted, monomeric	49
MUC11-12	Colon	Membrane-bound	21
MUC13	Colon, trachea (kidney, small intestine, appendix, stomach)	Membrane-bound	20
MUC16	Mullerian duct (embryonic, developing into reproductive structures)	Membrane-bound	50
MUC17	Small intestine, colon, (stomach), duodenum	Membrane-bound	22
MUC19	'*In silico*'		51

Table 2. Tissue distribution of mucins. Organs in parentheses represent low levels

Mucin glycosylation

The carbohydrate structures present on mucosal surfaces vary according to cell lineage, tissue location, and developmental stage. In addition, they undergo changes during malignant transformations, act as receptors for microorganisms and possibly play a role in evasion of pathogenic bacteria. The massive *O*-glycosylation of the mucins protects them from proteolytic enzymes and induces a relatively extended conformation.[52] The oligosaccharides are clustered into heavily glycosylated domains (typically 600-1200 amino acids long) separated by shorter non-glycosylated regions.[52] The extended conformation causes the molecules to occupy large volumes, and the secreted oligomeric mucins occupy domains equivalent to those of small bacteria.[52] The size of the polymer is an important factor in the formation of entangled gels, and cleavage of disulfide bonds will disintegrate mucus.[11] The extended conformation of the membrane-associated mucins enables them to extend far above the cell surface,[52] most likely further into the cell environment than most of the other glycoproteins comprising the glycocalyx. The *O*-linked glycans contain 1-20 residues, which occur both as linear and branched structures. The carbohydrate chain is initiated with an *N*-acetylgalactosamine (GalNAc) linked to serine or threonine and is elongated by the formation of the so-called core structures (there are 8 cores structures referred to as cores 1-8) followed by the backbone region (type-1 and type-2 chains).[53] The chains are terminated by *e.g.* fucose (Fuc), galactose (Gal), GalNAc or sialic acid residues in the peripheral region, forming histo-blood-group antigens such as A, B, H, Lewis a (Le^a), Lewis b (Le^b), Lewis x (Le^x), Lewis y (Le^y), as well as sialyl-Le^a and sialyl-Le^x structures (figure 5). Sulfation of Gal and *N*-acetylglucosamine (GlcNAc) residues causes further diversification. In addition to the *O*-linked glycans, mucins are substituted by a smaller number of *N*-linked oligosaccharides which have been implicated in folding, oligomerization (MUC2) or surface localization (MUC17).[54-56] The carbohydrate structures present depend on the glycosyl transferases expressed in the cells, *i.e.* by the genotype of the individual. The H1 structure is made by the *secretor* (*Se*) gene product, and the majority (80% of Caucasians, all South American Indians and Orientals) carry this structure and are thus referred to as 'secretors'.[57] Individuals may also express the *Lewis* (*Le*) gene (90% of the Caucasian population), and provided that they are also secretors will then express the Le^b structure on H1.[57, 58] If they are non-secretors, Le^a will be expressed on type-1 chains (Figure 5).[57, 58] The terminal structures of mucin oligosaccharides are highly heterogeneous and vary between/within species and even with tissue location within a single individual. Possibly, this structural diversity allows us to cope with diverse and rapidly changing pathogens, as reflected by the observation that susceptibility to specific pathogens differs between people with different histo-blood groups.[59] In addition, glycosylation changes occur during infection/inflammation, as in the rat small intestine during infection with the parasite *Nippostrongylus brasiliensis*,[60] and in individuals with cystic fibrosis or chronic bronchitis.[61] *H. pylori*-infected individuals have been found to have higher levels of sialyl-Le^a than non-infected ones,[62] however it is not known whether this difference is a predisposing factor for infection or a consequence thereof.

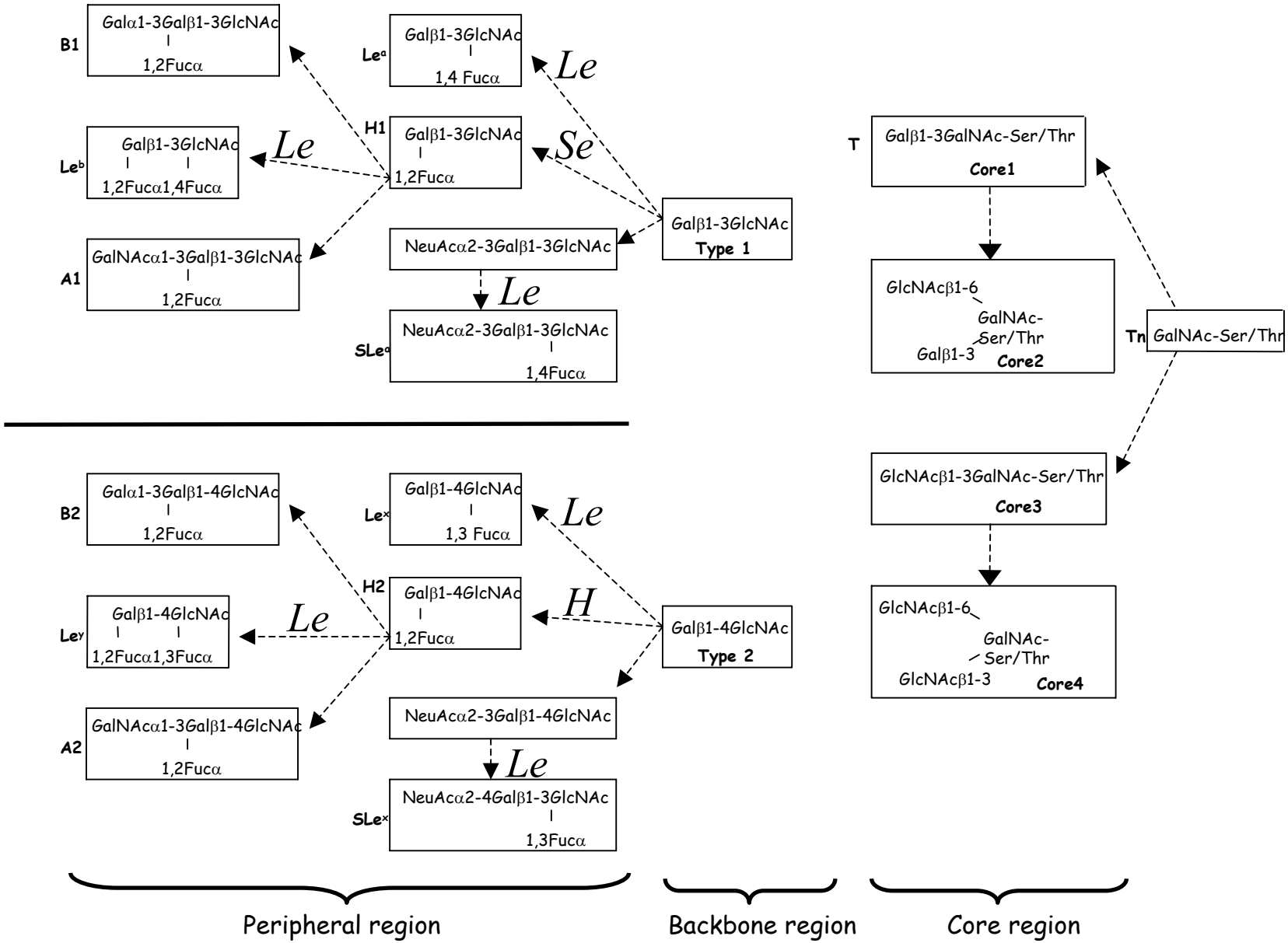


Figure 5. Schematic drawing of some oligosaccharide structures present on mucins. (Adapted from Herrmann, [53] with permission). The enzyme encoded by *H* works preferentially on type 2 chains and the enzyme encoded by *Se* on type 1 chains, although some overlap occurs.[57, 58]

Gastric mucins

In the stomach, MUC5AC and MUC6 are the major secreted mucins, the former being produced by the surface epithelium and the latter by the glands.[63] The membrane-associated MUC1 mucin has also been identified in the stomach.[40] Both MUC5AC and MUC6 are large oligomeric mucins that occur as distinct glycoforms. The molecular mass of MUC5AC subunits is 2-3x10^6 Da,[17] and MUC5AC is highly oligomerized, containing at least 18 subunits.[64] This mucin has an extended conformation,[64] and the N- and C-terminal ends show homologies to the von Willebrand factor (vWF). MUC6 has only been partially sequenced, but has a long TR and C-terminal similarities with the vWF.[65] The Le^a and Le^b blood-group antigens mainly appear in the surface epithelium, whereas the Le^x and Le^y antigens are expressed in mucous, chief and parietal cells of the glands.[63, 66] Thus, the Le type-1 structures co-localize with MUC5AC whereas Le type-2 structures co-localize with MUC6. However, some reports indicate that this distribution is not always that distinct.[66, 67] The mucins from a healthy human stomach express low levels of sialic acid and sulfate, and are therefore predominantly neutral.

Mucins in the diseased stomach

In gastric precancerous lesions and cancer, altered expression of MUC5AC, MUC6, MUC2 and MUC5B has been described,[68] with MUC2 being a marker for intestinal metaplasia. MUC1 is often over-expressed in epithelial cancer and has been implicated in adhesion and metastatic processes. Over-expression of MUC1 correlates with poor prognosis in a variety of cancer types.[69] Glycosylation of cancer-associated MUC1 is incomplete/truncated (4-6 sugar units), exposing internal sugar units and naked peptide sequences that are hidden on the normal mucin.[69] Expression of aberrantly glycosylated MUC1 correlates (whereas MUC5AC inversely correlates) with depth of invasion, tumor stage and lymph node metastasis, and patients with gastric tumors with a $MUC5AC^-/MUC1^+$ phenotype show low survival rate.[70] In *H. pylori* infected adults, aberrant MUC6 expression in the surface epithelium has been observed,[71] and the highly glycosylated 'ectodomain' of MUC1 seems to disappear from the luminal gastric surface.[38] In cancer, loss of correct tissue architecture/topology may allow mucins to be expressed and secreted on all surfaces of the epithelial cells, and soluble mucins may then enter the circulation. Mucins appear to be the major carrier of altered glycosylation in carcinomas,[72] and incomplete glycosylation, leading to expression of Tn and T antigens, and/or sialylation/sulfation is common.[72] Sialyl-Le^x and sialyl-Le^a are frequently over-expressed in carcinomas, and expression of these antigens by epithelial carcinomas correlates with tumor progression, metastatic spread and poor prognosis.[72] In most gastric tumors, acidic mucins predominate.[73] A shift in gastric mucin production may be detected in the diseased stomach before morphological changes are evident and could thus provide a valuable diagnostic tool.

Pathogen strategies for invasion/escape of host defense

The outcome of a persistent colonization can be symbiosis, commensalism or parasitism. The ability of bacteria to adhere, invade, evade host defenses and cause tissue damage is largely due to their ability to produce colonization and virulence factors. Colonization factors can, for example, be involved in adherence, iron acquisition and motility.[74] In addition, bacteria can have virulence factors that damage the host or undermine host defense, such as toxins and proteolytic enzymes.[75] To escape the host defense, pathogens can use sequestration (*i.e.* formation of a physical barrier against the host), humoral evasion strategies (*e.g.* expression of poorly immunogenic antigens or antigenic variation, mimicry and masking) and cellular evasion mechanisms (*e.g.* killing of phagocytes, inhibition of chemotaxis or phagocytosis, occupation of a 'safe' intracellular space, resistance to intra-vacuolar killing and suppression of cellular responses by cytokine manipulation).[76] Bacteria encounter a large variety of environmental situations, including changes in temperature, osmotic pressure, pH, oxygen and nutrition levels, and are highly competent in adapting to a changing environment.

Adherence to host cells

To colonize mucosal surfaces and invade the host, microbes commonly exploit host cell structures. Bacterial adhesion to host cells is thought to be mediated by hydrophobic interactions, cation-bridging (*i.e.* divalent cations counteracting the repulsion of the negatively charged surfaces of bacteria and host) and receptor ligand binding. One of the most extensively studied mechanisms of adhesion is the lectin and its corresponding glycosylated receptor. Bacteria may have multiple adhesins with different carbohydrate specificities, and modulation of surface receptor density, kinetic parameters, or topographical distributions of these receptors on cell membranes regulate adhesion. Binding is usually of low affinity, but clustering of adhesins and receptors cause multivalency effects. Fimbriae (or pili), outer membrane proteins and cell wall components (*e.g.* lipopolysaccharides, LPS), may all function as adhesins.[74] Adhesion can effect the bacteria by stimulation/inhibition of growth as well as induction of other adhesive structures and proteins required for invasion whereas effects of adhesion on host cells can be altered morphology, fluid loss, induction of cytokine release, upregulation of adhesion molecules and apoptosis. However some bacteria, as for example members of the normal microflora such as Enterococci and Lactobacilli, can colonize without any apparent effects.[8]

Host-microbe communication

Eukaryotic cells and bacteria communicate through a wide variety of signals such as toxins, metabolites, hormones, antibacterial peptides, enzymes and surface structures. There are 2000-5000 signal transduction proteins on the average mammalian cell, and a large number of lipid mediators are also involved in cell-cell and intracellular communication. In addition, bacteria use complex cell-cell and intracellular pathways to communicate, and have the capacity to utilize eukaryotic signaling pathways during infection. One example of how a pathogen has 'manipulated' its host to its advantage

is *Bacteroides thetaiotaomicron* that induces fucosylation of mucin oligosaccharides which the bacteria then uses as a nutritient.[77]

Helicobacter pylori

Spiral gram-negative bacteria have been observed in the human stomach.[78] Of the infectious agents causing gastric pathogenicity, *H. pylori* is the most common one, however *Helicobacter heilmannii* can cause milder forms of gastritis.[79, 80] In addition, other pathogens such as *Mycobacterium* spp., Herpes and Cytomegalovirus, fungi and parasites can infect/affect the gastric mucosa during special circumstances such as immunosuppression.[81]

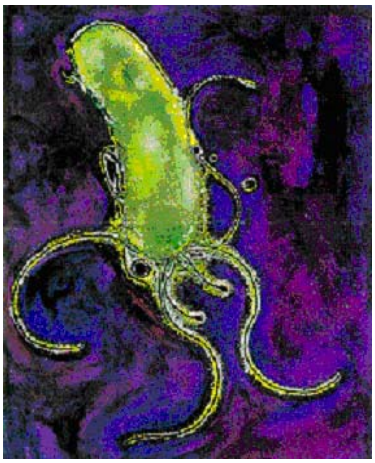


Figure 6. Image of *H. pylori* printed by permission of ÄRZTLICHE PRAXIS. *H. pylori* is a Gram-negative, rod/spiral-shaped micro-aerophilic bacterium,[82] that can cause peptic ulcer disease and gastric cancer.[83 84] The microbe is well adapted to the gastric mucus niche, having long whip-like flagella facilitating locomotion through the mucus layer. In addition, surface-bound urease that catalyzes the hydrolysis of urea to ammonia and carbon dioxide allows the microbe to 'neutralize' its microenvironment. Flagella, urease, adhesins, and genes encoding proteins with a predicted function in chemotaxis are essential for *H. pylori* colonization of laboratory animals.[85, 86] Furthermore, superoxide dismutase and catalase protect the microbe from oxidative damage.[86] Strains vary in pathogenicity, and the Cag pathogenicity island (PAI) that comprises approximately 30 genes associated with pathogenicity is present in pathogenic strains but not in avirulent ones.[82, 86] Some of these genes have close sequence similarities with a type IV secretion system, which provides a mechanism for transfer of bacterial proteins into eukaryotic cells.[82] Strains with mutations in the *ureB*, *pldA* and *fliQ* of the CagPAI induce less apoptosis in a gastric carcinoma cell line (AGS) than their parent strain.[87] In addition, the vacuolating cytotoxin encoded by *VacA* causes vacuolation of epithelial cells *in vitro*.[82]

H. pylori and mucins

During natural infection, *H. pylori* is mainly found in the gastric mucus layer,[88] although some (less than 1%) are also attached to the surface of, or found within, epithelial cells and the lamina propria.[86, 89] The highly diverse mucin glycosylation presents multiple potential binding motifs for *H. pylori.* The microbe binds to human and pig mucins,[90-92] and co-localization of MUC5AC producing cells and *H. pylori* has been shown in morphological investigations.[93, 94] In addition, *H. pylori* binds to the 'mucin like' salivary agglutinin.[95] The proportion of oligomeric gel-forming mucin in the adherent mucus layer is reduced in *H. pylori* positive individuals.[96] Mucin synthesis is partially inhibited in a gastric adenocarcinoma cell line (KATO III) when incubated with *H. pylori*, although no effect was found on mucin secretion.[97, 98] In contrast, mucin secretion was decreased in a colonic cancer cell line (Cl. 16E) and

in rat gastric mucosal cells.[99, 100] This discrepancy further stresses the need for appropriate models to study the effects of *H. pylori* on mucins.

H. pylori and the extracellular matrix (ECM)

H. pylori interacts with heparin and heparan sulfate with a binding maximum at pH 4-5 and with laminin (via 3-sialyllactose) and vitronectin at neutral pH.[101-104] In the healthy stomach, some of these ECM components are most likely not available for *H. pylori* binding early in the colonization process, but may be exposed when the normal tissue architecture is compromised. In addition, *H. pylori* binds to plasminogen, and after 'activation', plasmin may degrade ECM components.[105]

H. pylori adhesins

The genome of *H. pylori* codes for a large number (32) of outer membrane proteins,[106] and at least five different adhesins (see below) have been implicated in *H. pylori* adherence. Different adhesins may mediate adherence to various sites in the stomach, or in other reservoirs such as the oral cavity.

The blood-group binding adhesin (BabA) recognizes the Le^b and H-type-1 structures, and Le^b mediates attachment of *H. pylori* to the human gastric mucosa.[107] The majority of the *H. pylori* strains investigated carry the *BabA2* gene encoding the BabA adhesin.[108, 109] Colonization with *H. pylori* strains expressing the BabA adhesin correlates with development of severe gastric disease,[109, 110] and presence of the BabA2 gene in the bacterial genome has been shown to be a pathogenesis factor in the development of adenocarcinoma.[109]

The sialic acid binding adhesin (SabA) is encoded by the JHP662 gene.[111] SabA recognizes the sialyl-Le^x and sialyl-di-Le^x structures, and 43% of the SabA positive strains also bind sialyl-Le^a.[111] Of 95 Swedish clinical isolates, 39% bound sialyl-Le^x, and the prevalence of this adhesin was higher in CagA positive than in CagA negative strains.[111] Most (80%) of SabA positive strains are also BabA positive.[111] SabA positive *BabA1A2* mutants adhere to inflamed tissue, but not to healthy gastric mucosa.[111] Neuraminyl-Lactose-Binding Adhesin (NLBH) binds to *N*-acetylneuraminyl-(α2,3)-lactose (3-sialyllactose), and consequently has a very similar specificity to that of SabA.[112] Although some of the data reported concerning the gene(s) encoding SabA and NLBH are conflicting, a recent report indicates that these adhesins are actually the same.[113]

The *H. pylori* neutrophil activating protein (NAP) binds to sulfated carbohydrate structures such as sulfo-Le^a, sulfated glycosphingolipids, the NeuAcα3Galβ-4GlcNAcβ3Galβ4GlcNAcβ sequence from *e.g.* human granulocytes, and the high-molecular-mass salivary mucin at neutral pH.[114, 115] In addition, strain NCTC11637 binds to sulfatides at neutral pH, and binding was inhibited by sulfated glycoconjugates such as heparin and pig gastric mucin.[116] *H. pylori* NAP induces neutrophil adhesion to endothelial cells.[112]

H. pylori lipopolysaccharides substituted with Le^x antigen have also been implicated in adhesion, although the corresponding host lectin remains unknown.[117] Anti- Le^x antibodies have been found to label both the microbes and associated gastric epithelial adhesion pedestals.[112] Most (80 %) *H. pylori* strains express Le^x and/or Le^y structures on their lipopolysaccharides.[118]

Adherence-associated lipoprotein A and B (AlpA and AlpB) have a function in adherence as mutations/deletions in *AlpA* or *AlpB* reduce binding to gastric sections.[119] This binding is different from the BabA-dependent adherence.[119]

Prevalence and consequence of *H. pylori* infection

H. pylori infection is one of the most common bacterial infections in the world, colonizing approximately half the population.[82] Colonization usually occurs before the age of ten,[82] and once established within the gastric mucosa, the bacterium can persist for life, although transient infection occurs in a few individuals.[86] Person-to-person contact is believed to be the mode of transmission since prevalence is higher among institutionalized individuals, in family members of infected children, in families with many children and in developing countries.[82] *H. pylori* infection is associated with gastric/duodenal ulcers and gastric cancer,[83] and the WHO has classified *H. pylori* as a class 1 carcinogen.[84] Out of the infected half of the population, 15% are estimated to develop symptoms and 3% gastric cancer.[120] Although *H. pylori* is the cause of both gastric cancer and duodenal ulcer, the development of these conditions appears to be mutually exclusive, and duodenal ulcer is considered a protection factor against gastric cancer.[82] *H. pylori* infected individuals have a higher level of pro-inflammatory cytokines in the gastric mucosa compared to uninfected individuals.[82, 121, 122] The intense humoral and cellular immune responses associated with gastric colonization are usually unable to eradicate *H. pylori* and may play a major role in the morbidity of the disease. *H. pylori* and inflammatory cells infiltrating the mucosa produce cytotoxic agents such as reactive oxygen metabolites, ammonia and cytotoxins, which all cause damage to DNA and epithelial cells. In addition, *H. pylori* may cause excessive cell replication, removal of the protective mucus layer (allowing toxic substances to come into direct contact with the epithelial cells),[123] and decrease the mismatch repair genes in epithelial cells,[82] possibly contributing to carcinogenesis. Thus, gastric carcinomas may be caused by the converging effects of host inflammatory by-products causing mutational events in gastric epithelial cells and direct effects on gastric cells by *H. pylori*.

Infection with *H. pylori* may have several outcomes

Infection with *H. pylori* may go unnoticed or lead to gastric/duodenal ulcer, lymphoma or carcinoma,[123] and since most infected individuals show no clinical symptoms, the pathological process must be influenced by host/environmental factors in addition to the genotype of the infecting strain. In the majority of infected individuals *H. pylori* infection is antrum predominant, and acid production by the largely unaffected corpus is enhanced leading to an increased risk of DU. In a minority (individuals with low acid output) infection is corpus predominant, and

infection is associated with progressive gastric atrophy, subsequent hypochlorhydria and an increased risk of gastric cancer.[86] The Mongolian gerbil, which is the only rodent to develop gastric cancer after *H. pylori* infection in the absence of additional carcinogens, has a 15 times lower gastric acid secretion than other rodents.[82] Hypochlorhydria permits infection with other bacteria that may enhance the production of carcinogenic (*e.g. N*-nitroso) compounds.

The host secretor status in relation to gastric disease has been studied intensively. Although conflicting results on the relationship between secretor phenotype and *H. pylori* status or PUD have been reported,[124, 125] the majority of studies have demonstrated that non-secretors (who are devoid of Le^b epitopes) have a higher risk of developing gastro-duodenal disease than secretors.[126-129] However, the Le^b host phenotype *per se* does not seem to be associated with *H. pylori* infection,[125, 127, 130] although Taiwanese patients who express Le^b have a higher *H. pylori* density than those who do not, and *H. pylori* density in such patients increases with Le^b expression.[131]

Stress is another factor that may work in conjunction with *H. pylori* or cause ulcers through alternative pathways. People exposed to disasters as well as prisoners of war have increased occurrence of ulcers, and wound healing is slower in stressed individuals.[132] In addition, the prevalence of stress-associated gastric lesions is 52-100% of patients in intensive care units.[133]

Histo-pathological consequences of *H. pylori* infection

In antrum and cardia, *H. pylori* can grow unimpeded by acid, which explains why the majority of the bacteria are found in these regions. In contrast, *H. helmanii* is, at least in the rhesus monkey, found in all regions of the stomach, often colonizing even the parietal cells.[134]

H. pylori gastritis

During colonization, *H. pylori* penetrates the mucus layer and multiplies in close proximity to the surface epithelium causing acute neutrophilic *H. pylori* gastritis. The epithelium responds to infection by degenerative changes such as mucin depletion, cellular exfoliation and compensatory regenerative changes, hypochlorhydria, and production of cytokines such as IL-1β, IL-8 as well as tumor necrosis factor alpha (TNF-α).[135] In the acute phase, innate and natural immunity (neutrophils and macrophages/monocytes) rather than specific immune reactions dominate.[135] In the majority of individuals, there is a gradual accumulation of lymphocytes, plasma cells, B-cell proliferation and mucosa-associated lymphoid tissue, which turns the acute neutrophilic gastritis into an active chronic gastritis. The principal features of chronic gastritis comprise surface epithelial degradation, foveolar hyperplasia, hyperemia, lamina propria edema and atrophy.[13] In some subjects, dysplasia and intestinal metaplasia may appear.[13]

Duodenitis

In chronic duodenitis there is an increase in chronic inflammatory cells in the lamina propria and gastric metaplasia frequently occurs.[14] The degree of gastric metaplasia in the duodenum is closely associated with active chronic gastritis and the presence of *H. pylori* in the stomach.[136] The increased acid secretion, occurring in the majority of *H. pylori* infected stomachs, probably contributes to the increased duodenal acid load that may cause gastric metaplasia in the duodenum. In turn, gastric metaplasia allows *H. pylori* to prosper in this region.

Peptic ulcer disease

Peptic ulcer affects one in ten people and *H. pylori* infection, smoking, alcohol, stress as well as non-steroid anti-inflammatory drugs are important contributors to the development and perpetuation of the disease.[137] Gastric ulcers are usually preceded by corpus dominant or pan-gastritis, with *H. pylori* colonization of the corpus and low acid output.[82] Duodenal ulcers are more common than gastric ulcers. Patients with duodenal ulcers have on average a greater capacity for acid secretion than healthy people. Ulcers heal when acid is neutralized, and *H. pylori* eradication helps healing and prevents recurrence of ulcers.[136, 138]

Gastric neoplasia

Gastric cancer is the third most common cancer worldwide, and the second most common cause of cancer-related deaths.[139] Most gastric carcinomas appear in a background of chronic gastritis. The well differentiated (intestinal) type of carcinoma is preceded by inflammation, then multifocal atrophy and intestinal metaplasia, and this series of changes is generally initiated by *H. pylori*.[140] The association between *H. pylori* and cancer seems to be restricted to non-cardia gastric cancers, whereas cardia and esophageal adenocarcinoma are associated with reflux esophagitis.[83] Reflux esophagitis may be more rare in infected individuals due to the reduction in gastric acidity associated with the disease.[83] Calculating with an odds ratio of 5.9 (lower odds ratios have also been suggested[139]), 65-80% of non-cardia gastric cancers may be attributed to *H. pylori* infection and thus potentially preventable.[83] Furthermore, *H. pylori* infection may cause B-cell lymphoma of the mucosa-associated lymphoid tissue, and 80% of the cases can be healed by eradication of the bacteria.[141, 142] In the USA, malignant lymphoma constitutes 5% of gastric malignancies.[82]

Models to investigate *H. pylori*-host interactions

Cancer cell-lines, organ cultures of gastric biopsies and whole animal models have been used to investigate *H. pylori*-host interactions. A large variability in response to this pathogen has been noted in different species, making the choice of model system a major consideration. Conflicting results have been obtained from cell-lines, and to date there are no gastric surface epithelial cell-lines available that maintain the normal cell polarity. The most commonly used laboratory animals such as rats and mice have drawbacks since *H. pylori* is mainly a primate-specific bacterium. The effects of *H. pylori* on the mouse are mild, and gastric cancer is not induced even after long-term exposure, although the mouse may develop chronic atrophic gastritis.[143, 144] *H. pylori* can colonize the guinea-pig and the Mongolian gerbil and cause a severe inflammatory response.[145] These small animal models are useful to study some aspects of *H. pylori* infection, and have the advantage of being relatively cheap. In contrast, primates are very similar to humans, and rhesus monkeys naturally have persistent *H. pylori* infection leading to loss of mucus, gastritis, gastric ulcers and even cancer. [146-149] The rhesus monkey can be experimentally infected with *H. pylori*, and individuals differ in their susceptibility to particular bacterial strains.[146, 147] Consequently, the rhesus monkey is affected by *H. pylori* in a similar way to man. In addition, the anatomy and physiology of the GI tract of the rhesus monkey, as well as the expression of blood group antigens such as A-B-O, M, Rh-Hr, Lewis and i, are very similar to that in human.[150] However, little is known about rhesus monkey gastric mucins and *H. pylori* adherence compared to human.

PRESENT INVESTIGATION

H. pylori binding to human gastric mucins (papers I & II)

The aim of this part of the investigation was to identify the mucins from the healthy human gastric mucosa to which *H. pylori* bind. Eight strains (including those with the Le^b/H-type-1-binding BabA adhesins, isogenic mutants of such strains lacking the adhesin, and sialyl-Le^x-binding SabA-positive strains) were used to identify microbe-binding molecules from individuals with varying blood-group status, at a pH range from 1-7.4.

BabA positive strains bind to MUC5AC from Le^b-positive individuals and binding is inhibited by Le^b or H-type-1 conjugates

In Le^b-positive individuals, Le^b was present on the MUC5AC molecule. No association was found between *H. pylori* mucin binding and the H-type-2, Le^a, B and A blood-group antigens with any of the eight strains investigated. Strains with the SabA or AlpA adhesins demonstrated negligible binding to mucins from healthy tissue at neutral pH, indicating that SabA does not recognize sialic acid in this context, possibly because it is a different sialic acid-containing oligosaccharide structure than sialyl-Le^x. Consequently, the function of these adhesins is different from that of BabA, and *H. pylori* adherence to MUC5AC at neutral pH is affected both by host and microbe factors.

The BabA/Le^b-mediated receptor recognition of MUC5AC is strain and glycoform dependent

Binding with one of the Le^b-binding BabA positive strains (p466) to MUC5AC correlated well with the amount of Le^b as detected with the 2-25LE antibody. This Le^b antibody does not recognize the BLe^b structure, and reactivity of both this antibody and strain p466 with mucins from a B-positive individual was low compared to reactivity with an antibody (96FR2.10) recognizing the Le^b, BLe^b, ALe^b and H-type-1 structures and the second Le^b-binding BabA positive strain (CCUG17875) investigated. Thus, the BabA adhesin of strain p466 does not recognize the BLe^b structure as well as the Le^b structure, whereas the BabA adhesin of strain CCUG17875 does not discriminate between the two. Thus, the BabA adhesin(s) differ in their ability to recognize Le^b versus BLe^b.

Anion-exchange chromatography revealed three major MUC5AC glycoforms (Figure 7a, d and g) that differed in their microbe-binding properties. One of the Le^b-binding BabA positive strains (p466) bound to all three glycoforms in all Le^b-positive samples, whereas the other strain (CCUG17875) bound to all 3 glycoforms in one sample but only to the most charged one (glycoform 3) in the other samples (figure 7 c, f and i). Consequently, the Le^b structure appears to be a sufficient requirement for binding of strain p466, whereas the presence of this structure seems to be a necessary but not sufficient requirement for binding of strain CCUG17875 to MUC5AC. Thus, the BabA adhesins differ in their ability to recognize MUC5AC glycoforms carrying the Le^b structure and preferential glycoform binding (with possible inter-individual

variations in glycoform expression in the population) adds yet another level of complexity to mucin-microbe interactions in addition to the highly diverse oligosaccharide substitution.

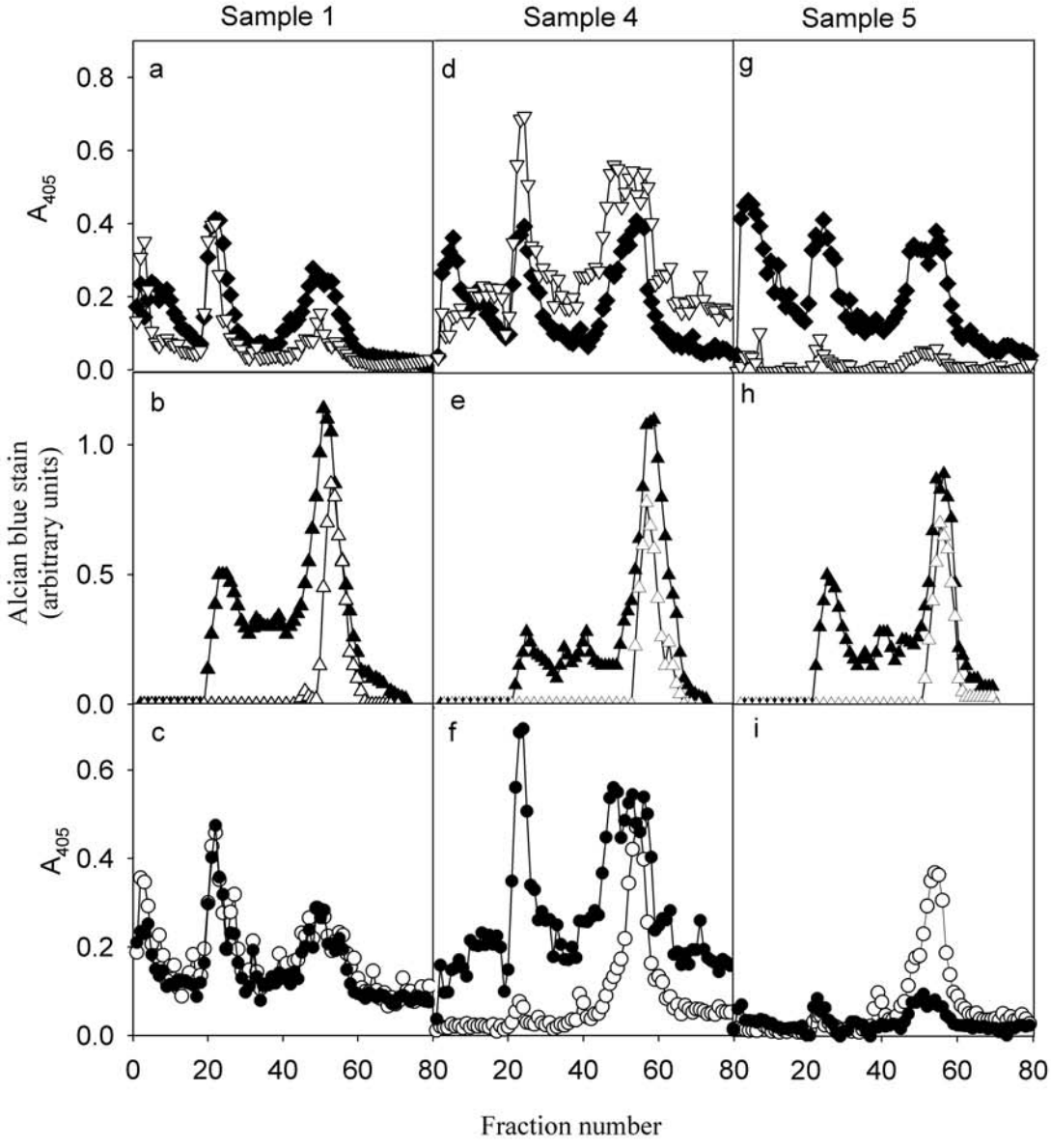


Figure 7. **Mucin glycoforms**. Mucin subunits were subjected to anion-exchange chromatography on a Mono Q HR5/5 column. Fractions were analyzed for MUC5AC (◆) and Le^b (2-25LE) (▽) (panel a, d and g) Alcian blue staining at pH 2.5 (▲) and pH 1.0 (△) (panel b, e and h) and binding with strains CCUG17875 (○) and p466 (●) (panel c, f and i).

At neutral pH, BabA positive *H. pylori* strains bind to a mucin-like molecule of smaller size than MUC5AC

In individuals expressing the Le^b structure, Le^b-binding strains bound to a component of 'typical' mucin density but of smaller size than MUC5AC. An antibody against MUC1 reacted with part of this low-molecular-mass material. However, the rest of the population may also be MUC1 since it is well known that antibodies against the MUC1 ectodomain are sensitive to glycosylation that may shield the epitopes, and possibly only a MUC1 subpopulation with a 'low' degree of glycosylation is recognized. This material reacted with an antibody (clone 96FR 2) that recognizes the H-type-1 structure in addition to Le^b, but not with the Le^b antibody, suggesting that the H-type-1 structure is present. Binding was inhibited by Le^b and H-type-1 HSA conjugates, with a higher concentration of H-type-1 than Le^b needed for the same level of inhibition. Thus, BabA positive *H. pylori* strains bind both H-type-1 on the putative membrane-bound MUC1 mucin and Le^b on secreted MUC5AC in the mucus layer. MUC5AC may thus function as an effective decoy for MUC1 since the BabA adhesin has a higher affinity for Le^b than for H-type-1 and MUC5AC is present in much larger amounts.

At acidic pH, *H. pylori* binding occurred to the most charged MUC5AC glycoform, a putative 'novel' monomeric mucin, a proteoglycan-like molecule and DNA

The glycoforms mentioned above may be described as neutral mucins (glycoform 1), sialomucins (glycoform 2) and a mixture of sialo- and sulfo- mucins (glycoform 3), with the sulfated species within glycoform 3 having a slightly higher charge than the sialylated ones (figure 7 b, e and h). In Le^b-negative samples, no binding was detected to glycoforms 1 and 2, whereas binding to glycoform 3 had a maximum at pH 3-4 for all strains. In the Le^b-positive samples, binding was similar for the non-Le^b-binding strains, whereas binding of the Le^b-binding strains to glycoforms 1 and 2 increased with pH, and binding to glycoform 3 was most pronounced at pH 3-5.

At pH 3, binding occurred to a putative 'novel' monomeric mucin, of higher density, charge and size than MUC5AC subunits. This component had a slightly higher density than MUC5AC and MUC6 in a CsCl density-gradient and was fragmented into a larger and a smaller unit after reduction. The larger unit has a greater hydrodynamic volume than the subunits of MUC5AC and MUC6 and is thus likely to be glycosylated whereas the smaller one may represent a cleavage fragment attached via disulfide bonds. The charge density of this 'high-density' component, as determined by anion-exchange chromatography, is slightly higher than the main population of glycoform 3, and corresponds to the sulfated sub-population of this glycoform. The component is present in very small amounts relative to MUC5AC and MUC6 as expected for a membrane-bound mucin present as a single molecular layer at the luminal plasma membrane in comparison with the secreted mucins that occur as both as a thick mucus gel and as highly condensed secretory granules.

At pH 3, all strains bound to DNA and to a component that could be degraded by chondroitinase ABC lyase (a putative proteoglycan) and this binding did not occur at pH 7.4. Binding to the latter component was stronger at pH 3, whereas binding to DNA was more pronounced at pH 5.

At acidic pH, no difference was detected in *H. pylori* binding to mucins, to the putative proteoglycan or to DNA between the strains investigated, including strains carrying/lacking the AlpA, SabA and BabA adhesins. This indicates that these particular adhesins are not responsible for binding at low pH. Furthermore, no differences in binding were detected between mucins carrying different blood-group structures. Consequently, the binding properties at acidic pH seem to be independent of host histo-blood group type as well as the genotype of the *H. pylori* strain.

The rhesus monkey as a model for *H. pylori*-host interactions (paper III)

The aim of this part of the investigation was to study the structure, tissue localization, histo-blood-group expression and *H. pylori* binding properties of gastric mucins from the rhesus monkey in order to evaluate whether this species is a suitable model for studies on *H. pylori*-mucin interactions.

The rhesus monkey produces mucins orthologues to human MUC5AC and MUC6 that have similar density, size, glycoforms, oligomeric structure and tissue localization to these mucins

After isopycnic density-gradient centrifugation, muc5ac and muc6 were found as a peak at 1.4 g/ml, and gel chromatography on both reduced and intact mucins revealed that muc5ac and muc6 from rhesus monkey are large oligomeric structures held together by disulfide bonds. Anion-exchange chromatography separated muc5ac and muc6 into three glycoforms, which were either not retained by the column (glycoform 1), eluted at the beginning of the gradient (glycoform 2) or more retained (glycoform 3). Sulfate was only found in glycoform 3 and the sulfated species had a slightly higher charge than the main population of this glycoform. Sialic acid was mainly found in glycoforms 2 and 3. In tissue sections, muc5ac localized to the surface/foveolar mucous cells and muc6 to the glands in antrum and cardia, whereas in corpus, muc5ac was found in the surface/foveolar epithelium and muc6 was present in mucous cells and intraluminal mucus in the neck region. In duodenum, the Brunner's glands were positive for muc6 and the goblet cells for muc2.

Rhesus monkey mucins are substituted with human-type histo blood-group antigens, and these structures have a similar expression pattern as in humans

The B, Le^b, Le^a, Le^x, Le^y, H-type-2, sialyl-Le^x, sialyl-Le^a, Tn and T antigens were detected on rhesus monkey gastric mucins. All Le^b-positive monkeys were also positive for Le^a, and these structures mainly localized to the surface mucous cells, although Le^b was also found in 10-70% of the antral glands. When present, sialyl-Le^a was located to the surface/foveolar epithelium, whereas no sulfo-Le^a was detected in any of the gastric specimens. Le^y was found in the glands of all monkeys both in

antrum and corpus, but rarely in the foveolar epithelium. Antibodies against Le^x and/or sialyl-Le^x stained the antral glands in the majority of monkeys, and in a few individuals also the foveolar epithelium. Thus, the Le-type-1 and Le-type-2 structures are mainly detected in the surface and glands respectively. The relative amount of the carbohydrate structures varies between individuals.

***H. pylori* binds rhesus monkey gastric mucins via at least 3 different mechanisms; to host Le^b and related structures via BabA, to host sialyl-Le^x via SabA and to sialylated host structures at low pH**

At neutral pH, *H. pylori* strains expressing the BabA adhesin bind rhesus monkey gastric mucins via the Le^b or H-type-1 structures on muc5ac and on a putative small monomeric mucin. The BabA-positive *H. pylori* strain bound to all 3 glycoforms, but mainly to glycoforms 2 and 3. Statistical correlation parameters between binding of antibodies/lectins and *H. pylori* strains calculated for the anion-exchange chromatography fractions showed that the correlations between reactivity of the BabA positive strain CCUG17875 at pH 7.4 and Le^b or the antibody recognizing the Le^b, BLe^b, ALe^b and H-type-1 structures were significantly stronger ($p = 0.001$) than to sialyl-Le^x, Peanut agglutinin (PNA, recognizing Galβ1,3GalNAc), or *Maakia amurensis* II lectin (MAL, recognizing NeuAcα2,3Galβ1,4GlcNAc) reactivity. This binding occurred to muc5ac rather than to muc6 ($p = 0.001$). Gel chromatography of material with 'typical' mucin density revealed an additional smaller apparently monomeric mucin (insensitive to reduction) that BabA positive strains also bound to. Binding to both muc5ac and this putative small mucin was inhibited by Le^b and H-type-1 conjugates.

At neutral pH, the SabA-positive *H. pylori* strain bound to sialyl-Le^x positive mucins, although seemingly with less efficiency than the BabA dependent interaction described above. Binding with the SabA-positive, BabA-negative, mutant (*babA1A2*) coincided with sialyl-Le^x but not with sialyl-Le^a, and this binding was approximately 25% of that of the BabA-positive ones. Removal of the sialic acid moieties with neuraminidase completely abolished binding. Binding with the SabA-positive mutant was mainly detected to glycoforms 2 and 3, and the correlation of adhesion with sialyl-Le^x was significantly stronger ($p < 0.001$) than to Le^b, H-type-1, MAL, PNA or 'carbohydrate' (periodate-oxidizable structures).

At acidic pH, *H. pylori* binds mucins carrying sialylated structures such as sialyl-Le^x and sialyl-core-type-2. *H. pylori* bound to glycoform 2, to a component reacting with the *Maakia amurensis* lectin eluting between glycoforms 2 and 3 and to glycoform 3. Binding was inhibited by DNA and dextran sulfate, and neuraminidase treatment abolished most of the binding. Binding did not occur to the sulfated subpopulation of glycoform 3, indicating that charge is not the only requirement for adhesion. The statistical correlation of binding at acidic pH was similar to sialyl-Le^x and to MAL reactivity, and did not differ significantly. The correlations between binding with the SabA positive mutant at pH 7.4 and the SabA positive mutant at pH 3 are significantly different, suggesting that these constitute different mechanisms of binding.

Effects of *H. pylori* infection on host mucins (paper IV)

The aim of this part of the investigation was to determine the effects of *H. pylori* inoculation on gastric mucin apoprotein tissue localization, glycosylation and *H. pylori* adherence.

The tissue localization of the mucus-forming mucins remained unchanged during the 10-month observation period

muc5ac was located to the surface/foveolar epithelium of antrum and corpus, and muc6 to the antral glands and corpus mucous cells as well as secreted mucus in the neck region. muc2 and sulfo-mucins were not found in mucin-producing cells in the stomach in any of the monkeys.

Glycosylation changes occurred in all infected animals

Expression of sialylated Lewis antigens in the surface/foveolar epithelium was increased one week after infection, and was strongest before four months. The percentage of the sialyl-Le^a positive surface/foveolar epithelium markedly increased in all nine infected monkeys and the mean percentage of sialyl-Le^a positive cells in the persistently infected animals increased significantly. In seven of the nine infected monkeys, the levels decreased late during infection. The percentage of sialyl-Le^x positive surface/foveolar epithelium increased during the first week in persistently infected monkeys, and returned to pre-inoculation levels after 4-10 months in most animals. However, this increase did not reach a level of statistical significance ($p = 0.053$) due to the large inter-individual variability. Interestingly, sialyl-Le^x levels remained unchanged in the monkey that spontaneously cleared the infection. A significant correlation was found between sialyl-Le^x and sialyl-Le^a in antral surface/foveolar epithelium, and *H. pylori* density and gastritis score significantly correlated with sialyl-Le^x ($p < 0.001$ vs $p = 0.014$) and sialyl-Le^a ($p < 0.001$ for both) expression.

Le^a and/or Le^b expression transiently decreased in 5 out of 7 Le^b-positive animals one week after infection, but later increased. In addition, an initial decrease in fucosylation affecting structures other than Le^a/Le^b also seemed to occur since *in vitro* adherence with the *H. pylori* strain CCUG17875, which binds to fucosylated antigens such as Le^b and H-type-1, decreased in 7/8 infected monkeys, including the Le^b negative ones (see below). Two months after inoculation, the stain intensity had returned to pre-inoculation or higher levels. Le^a and/or Le^b expression also increased in all Le^a/Le^b-negative monkeys later during infection, and in some animals this increase tended to be strongest apically.

***In vitro* adherence of BabA- and SabA-positive *H. pylori* strains reflects the glycosylation changes**

In vitro adherence of the BabA-positive Le^b-binding strain CCUG17875 significantly ($p = 0.028$) decreased one week after inoculation. *In vitro* adherence to the mucosa of the transiently infected monkey initially decreased as in the persistently infected animals, but later increased as in the non-infected one with the increase preceding the

clearance of infection. No *in vitro* adherence was detected to the antral glands in any of the monkeys, although Le^b was detected in the glands of most animals, validating that the presentation of this structure is important for binding as shown in paper I. In Le^b-positive monkeys, *in vitro* adherence to the antral surface/foveolar epithelium was slightly higher before inoculation (mean 18 % vs 15%) and significantly higher after inoculation (1.8 times, p =0.007) than in Le^b-negative animals. However, adherence occurred in all individuals, indicating that structures other than Le^b (*e.g.* H-type-1) also mediate binding. *In vivo H. pylori* density, gastritis score and sialyl-Le^x levels significantly inversely correlated with *in vitro* adherence of Le^b-binding strain.

In vitro adherence with the SabA positive sialyl-Le^x binding *babA1A2* mutant to antral surface/foveolar epithelium significantly (p =0.015) increased in persistently infected monkeys and correlated with the levels of sialyl-Le^x (p <0.001) and sialyl-Le^a (p <0.001). *In vivo H. pylori* density and gastritis score significantly correlated with *in vitro* adherence of the SabA-positive strain. *In vitro* adherence of BabA- and SabA-positive strains progressed in opposite directions during infection (Figure 8), and adherence with these two strains inversely correlated (p =0.020). No *in vitro* adherence was detected with the AlpA-positive strain p1.

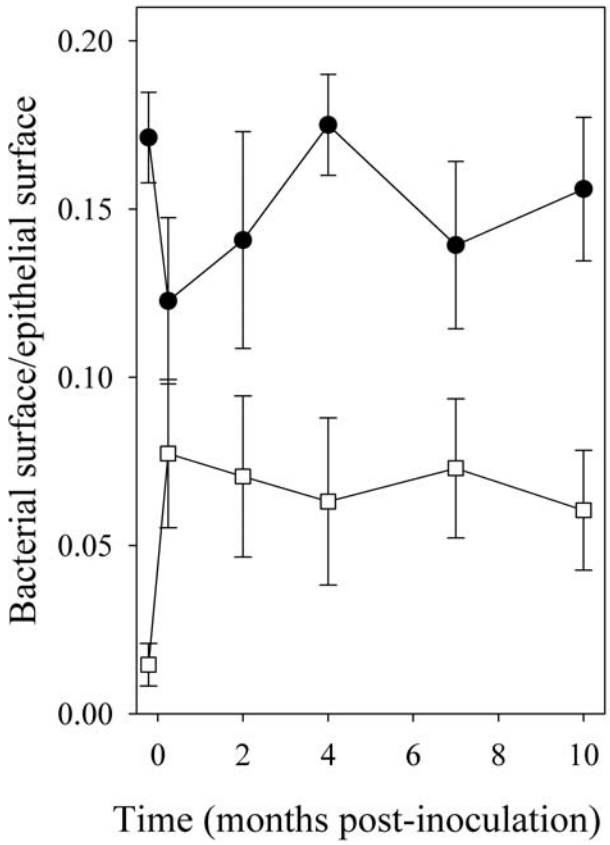


Figure 8. *In vitro* adherence of BabA- (●) and SabA-positive (□) strains progressed in opposite directions during infection.

The Le^b-positive monkeys were less affected by *H. pylori* than the Le^b-negative ones

After *H. pylori* inoculation, 8 of 10 rhesus monkeys developed persistent infection, with gastritis score increasing with *H. pylori* density score. However, the corpus gastritis score, the antrum *H. pylori* score and the corpus *H. pylori* score were approximately twice as high in Le^b-negative as in Le^b-positive persistently infected animals. In addition, the two monkeys that did not develop persistent infection were both Le^b-positive. Thus, although the number of individuals is too small to draw any general conclusions, both *H. pylori* density (figure 9) and gastritis score of Le^b-positive monkeys were lower than in the Le^b-negative animals. This suggests that attachment of *H. pylori* to Le^b-positive gastric mucin may protect against *H. pylori* infection by *e.g.* inhibiting attachment of the organism to underlying epithelial cells.

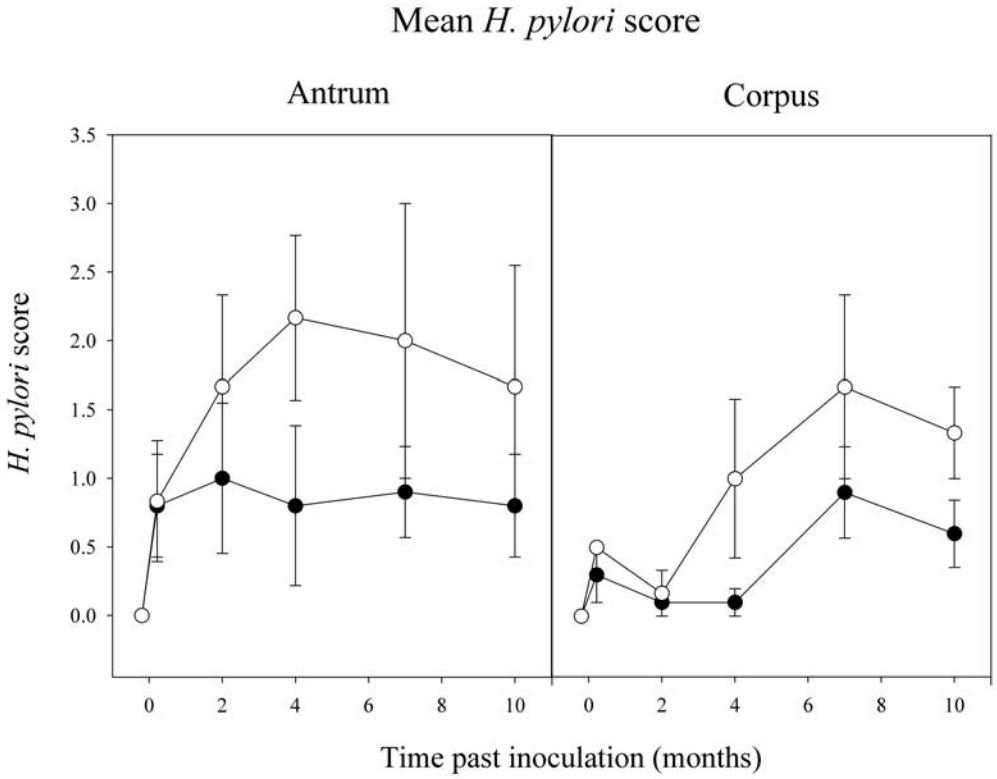


Figure 9. The mean *H. pylori* score was approximately twice as high in persistently infected Le^b-negative (○) as Le^b-positive (●) animals. The error bars represent standard error of the mean.

DISCUSSION

***H. pylori* binding to human gastric mucins**

Of the two major secreted gastric mucins MUC5AC and MUC6, binding via the Le^b-binding BabA adhesin occurred to MUC5AC. Although no experiments were performed to formally prove that *H. pylori* does not bind to MUC6, no observations were made suggesting that this is actually the case. In fact, binding correlated better with MUC5AC than with MUC6, both using biochemical binding assays (papers I & III) and *in vitro* adherence tests (paper IV). Mucin genes are polymorphic and the *O*-linked oligosaccharides are structurally highly diverse. Furthermore, gastric MUC5AC glycoforms differ in their ability to bind to *H. pylori* and the different strains differ in *their* mucin binding properties (paper I & II). The structure (Le^b) mediating adherence at neutral pH was present in all three glycoforms, suggesting that bacterial adhesins are influenced by the context in which their target structure appears, for example, by adjacent structures conferring steric constraints or charge repulsion. In contrast, binding to the putative membrane-bound MUC1 mucin seemed equal both in samples where MUC5AC binding was glycoform dependent and independent (paper II). Possibly, the microbes have developed mechanisms by which binding to the secreted mucins can be decreased while binding to the membrane-bound ones is retained in order to diminish the fraction of bacteria trapped in mucus that is removed from the stomach with gastric emptying.

Binding via the Le^b-binding BabA adhesin occurs at neutral pH and decreases with pH (paper II), which may allow the microbes to adhere at neutral pH and detach when the mucus gel is released into the acidic gastric juice. In principle, this would allow the bacteria to swim back into the mucus layer that has a pH compatible with survival. However, there are mechanisms that may prevent this from happening. For example, other large components could adhere to *H. pylori* in the gastric lumen. *H. pylori* binding to charged molecules at acidic pH seems to be a feature that is common to all strains studied (paper II). Although gastric mucins from healthy individuals are predominantly neutral, a portion belongs to the highly charged glycoform 3 (paper I & II). Furthermore, sialylated Le antigens increase after *H. pylori* infection (paper IV) and a decrease in the gastric juice acidic mucin content after eradication of *H. pylori* has previously been described.[151] Also in the rhesus monkeys infected with *H. heilmannii,* there was a tendency towards higher levels of sialyl-Le^x in the antral glands in *H. heilmannii* positive animals (paper III). Gastric mucins from pig [152] and rat[153] have also been shown to carry negative charges, suggesting that the proportion of acidic mucins in the human stomach is lower compared to other mammals. The human stomach is most likely less challenged by bacteria in the food than in other species and possibly, production of acidic mucins induced by bacterial challenge provides *one* mechanism to remove bacteria. Adherence to acidic mucins at low pH in the lumen might prevent the microbes from 'burrowing' into the mucus layer and thus retain them in an environment incompatible with survival. Consequently, the microbes would be removed with the gastric contents and the number of *H. pylori* in the stomach lowered.

Rhesus monkey gastric mucins are very similar to the cognate human ones
Expression and tissue localization of mucins and Lewis antigens, as well as mucin structure, density, glycoforms and *H. pylori* binding properties are similar in rhesus monkey and man (paper III). These observations indicate that the rhesus monkey is a good model for investigations concerning the role of gastric mucins in *H. pylori* colonization and persistence, and that results from studies in this species are likely to be relevant to human disease. Most animal models used for investigating *H. pylori* infection are different from humans regarding gastric anatomy, physiology and inflammatory responses, and contrasting results have emerged from studies using cell-lines. The physiology of the gastrointestinal tract of rhesus monkey resembles that of human, and the gastric mucins of the two species are very similar, suggesting that this animal model closely reflects *H. pylori* infection in man.

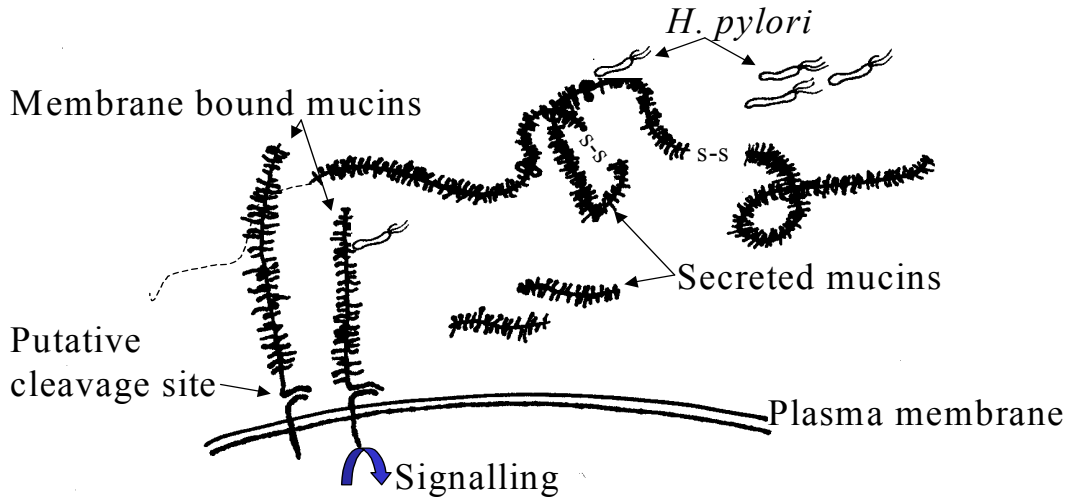


Figure 10. Binding to secreted mucins may protect against *H. pylori* by inhibiting attachment to the membrane-bound mucins or other structures on the underlying epithelial cells. Competition between *H. pylori* binding to membrane-associated and secreted mucins is likely to influence the outcome of the host-microbe interaction, since the membrane associated mucins have been shown to participate in signaling events.[154]

Competition between binding to membrane-associated mucins and secreted ones
In both man and monkey, adhesion occurred to a small apparently monomeric component of mucin density (paper II & III). In the human mucin preparations this component appeared, at least in part, to be MUC1 (paper II), and after infection fucosylation seemed to increase apically in some monkeys (paper IV). Other investigators have shown that the role of MUC1 could be dynamic in *H. pylori* infection since

reactivity with an antibody against the MUC1 exodomain is lost, indicating that the exodomain is either cleaved off or subjected to glycosylation changes.[38] In the non-infected stomach, the Le^b binding BabA positive strains bind both to Le^b on the secreted MUC5AC mucin and to H-type-1 on this putative membrane-associated MUC1 mucin (paper II). After infection, when fucosylation changes and Le^b seems to increase apically on the foveolar cells (paper IV), it is likely that this proportion is changed. MUC5AC may function as an effective decoy for *H. pylori*-MUC1 binding, as emphasized by the fact that BabA has a higher affinity for Le^b than for H-type-1 and that the MUC5AC mucin is present in much larger amounts than MUC1. Competition between *H. pylori* binding to membrane-bound and secreted mucins is likely to influence the outcome of the host-microbe interaction since membrane-bound mucins have the capacity for trans-membrane signaling (figure 10). Thus, the mucosal barrier, composed by the membrane-bound and secreted mucins is dynamic and responsive.

Glycosylation changes after infection

Although Le^a and Le^b expression initially decreased in the Le^b-positive animals after inoculation, there was a tendency to an increase later during infection (paper IV). In addition, in all three monkeys that were Le^b and Le^a negative before inoculation, Le^b and/or Le^a were detected after inoculation (paper IV). A similar effect is induced by some commensal bacteria (*e.g. B. Thetaiotaomicron*) that can signal to the host to produce more fucose on their glycans for the bacterium to use as nutrients.[77, 155] The increase in sialyl-Le^a and sialyl-Le^x expression in the surface/foveolar epithelium is most prominent during the first 2 months after inoculation (paper IV). Changes in levels of sialyl-Le^a before and after eradication have been reported, but to a lesser degree and only in 50% of specimens.[62] This could be explained by our finding that the change is time-dependent. Over-expression of sialyl-Le^a and sialyl-Le^x may correspond to 1) an increased expression of an α2,3-sialyl-transferase, competing with the fucosyltransferase for the same substrate, the terminal Galβ1-3GlcNAc and Galβ1-4GlcNAc chains (type 1 and 2 chains) and 2) an increased expression of α1,3- and α1,4-fucosyltransferase activity. The pro-inflammatory cytokine TNF (which is increased in *H. pylori* infection) increases the alpha 1,3-fucosyltransferase activity in cultured endothelial cells,[156] indicating that this could be a general response to inflammation. However, in our study correlations with sialyl-Le^a have a strong within-animal-correlation (R_w) and correlations with sialyl-Le^x a stronger between-animal-correlation (R_b) (paper IV). Thus, sialylation of Le^a and Le^x occurs as separate entities and is therefore not likely to be induced by the same mechanism, possibly because expression of sialyl-Le^x requires induction of the H-type-2 structure in the surface/foveolar epithelium. Glycosylation changes occur during infection/inflammation in the rat small intestine during infection of the parasite *Nippostrongylus brasiliensis*.[157] In individuals with cystic fibrosis (CF) or chronic bronchitis an increase in sialylation and sulfation of airway mucins is found, and respiratory mucins from CF patients have a higher affinity for *Pseudomonas aeruginosa* than most mucins from non-CF subjects. [61] During *H. pylori* infection in rhesus monkeys, glycosylation changes that differ between individuals and affect *H. pylori* adhesion targets occur (paper IV). Possibly, it is advantageous for the host that pathogen binding varies

between individuals since a certain pathogen would be unable to colonize an entire population. The personal repertoire of TR, glycosyl transferases, mucin glycoforms, and insult response is likely to affect individual susceptibility to different pathogens.

The host Le^b status influences *H. pylori*-host interactions

H. pylori density and gastritis score were lower in Le^b-positive than Le^b negative animals (paper IV). In addition, the animal that did not develop infection and the one that spontaneously cleared the infection were both Le^b-positive (paper IV). Although the number of individuals is too small to draw general conclusions, the Le^b-positive animals in our study were less affected by the *H. pylori* infection than the Le^b-negative ones. The observations that absence of Le^b is significantly associated with gastritis, and *H. pylori* density is significantly higher ($p = 0.045$) in Le^b negative children than in Le^b-positive ones support this notion (Sara Lindén, James Rick and André Dubois, unpublished observations). Most previous studies have found that non-secretors, who are devoid of the Le^b epitope, have a higher risk of developing gastroduodenal disease than secretors.[126-129] However, the Le^b host phenotype is not associated with *H. pylori* infection, although *H. pylori* infected patients in Taiwan who express Le^b have a higher *H. pylori* density than those who do not, and *H. pylori* density increases with Le^b expression.[127, 130, 131] These seemingly contradictory results may be explained by our results that Le^b expression increases both in Le^b-positive and Le^b-negative monkeys later during infection (paper IV), since studies in humans do not account for *H. pylori* induced changes. Furthermore, *H. pylori in vitro* adherence to foveolar epithelium with the Le^b binding strain was higher in Le^b-positive monkeys than in Le^b-negative ones, whereas *in vitro* adherence of the sialyl-Le^x binding strain was higher in Le^b negative monkeys (paper IV). Thus, the receptors for both the BabA and SabA adhesins indeed differ between Le^b-positive and negative individuals.

The fact that *H. pylori* infection can modify host glycosylation within mucus producing cells without modifying mucin expression or tissue localization implies that the change is not due to the expression of a 'new' component but to an altered glycosylation of MUC5AC. Bacterial challenge of mucosal surfaces triggers transient changes in host glycosylation within mucus producing cells, and this rapid response influences the structure of putative colonization targets. In turn, these structural changes of demonstrated colonization targets probably result in bacterial adaptation and selection of the strains that are best adapted to a particular host. These host-bacterial interactions may determine the outcome of the infection, *i.e.* persistence or transience, and perhaps whether or not disease develops.

OVERALL CONCLUSIONS

- *H. pylori* binding to gastric mucins at neutral pH is dependent on *H. pylori* strain and host blood-group expression. In contrast, all *H. pylori* strains investigated have similar binding properties at acidic pH.

- Binding to secreted mucins and putative membrane-bound ones occurs both at neutral and acidic pH.

- The BabA adhesins differ in their ability to recognize MUC5AC glycoforms carrying the Le^b structure.

- Expression and tissue localization of mucins and Lewis antigens as well as mucin structure, density, glycoforms and *H. pylori* binding properties are similar in rhesus monkey and man.

- The host responds rapidly to bacterial challenge by changing the expression of carbohydrate structures used by the microbe for adhesion. Microbe challenge of mucosal surfaces may thus trigger changes in host glycosyl transferase expression that influence the structure of putative colonization targets.

FUTURE OUTLOOK

In my studies, I have found that *H. pylori* binds to secreted mucins and putative membrane-bound ones both at neutral and acidic pH, although via different mechanisms. Since competition between binding to secreted mucins and membrane-bound ones with signaling potential is likely to influence the outcome of the host-microbe interaction, it is important to elucidate the roles of these structures relative to each other during colonization, and to find out what the consequences of binding to the membrane-bound mucins are, if any. Furthermore, glycosylation of the gastric mucosa was altered after infection, and some changes seemed to occur apically. Such glycosylation changes are likely to influence the competition between binding to secreted mucins and the membrane-bound ones. Consequently it is essential to investigate on what structures/mucins the glycosylation changes occur, and how the changes influence *H. pylori* adhesion to these structures.

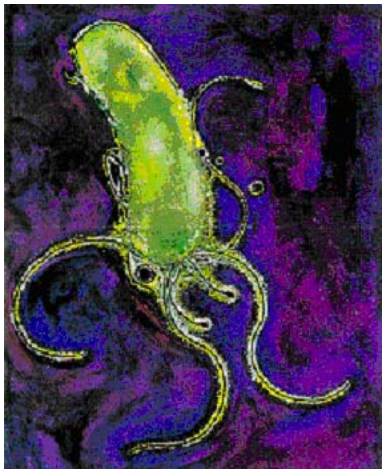
After the discovery of *H. pylori* 15 years ago, the focus of research on gastric ulcers moved from stress to this bacterium. However, only a subset of infected individuals develops symptoms, and often decades after infection indicating that environmental factors are important for the pathological process. A reduction in acidic gastric mucin biosynthesis has been observed in stressed rats,[158] and the amount of acidic mucins in the gastric juice is suppressed for at least three days in irradiated rhesus monkeys.[159] Since ionizing radiation produces free-radicals and oxidative damage has been implicated the development of stress ulcers,[160] the production of acidic mucins may be decreased in periods of stress in primates too. Thus, the putative elimination of *H. pylori* through binding to acidic mucins may decrease during periods of stress, which would lead to an increase in *H. pylori* bacterial load, and in turn could be a contributing factor for the correlation of stress and gastritis/gastric ulceration. Thus, it is time to bring back the stress aspect and investigate the converging effects of *H. pylori* and stress**.**

POPULARIZED SUMMARY IN SWEDISH

Magsårsbakterien *H. pylori* binder till och förändrar magens slemhinna

Bakterierna är i majoritet i kroppen!

Människans 10 biljoner celler lever i harmoni med 100 biljoner bakterier som har koloniserat någon av kroppens ekologiska nischer utan att göra skada. Av alla bakterier kroppen möter utgör bara en bråkdel något hot, och trots att kroppen möter sjukdomsalstrande bakterier flera gånger om dagen är det sällan man blir sjuk. Kroppens yta täcks på utsidan av huden (ca 2 m^2) och på insidan av slemhinnorna som tillsammans har en betydligt större yta (ca 400 m^2) eftersom de är veckade. Infektion påbörjas när en av dessa ytor blir koloniserad av bakterier. Kroppens yta skyddas av cellavstötning och en vänlig bakterieflora. Slemhinnorna är mer lättgenomträngliga än huden eftersom de även har funktioner som utsöndring av magsyra och enzymer som bryter ned födan samt upptag av näringsämnen från födan. Därför skyddas de dessutom av ett slemlager som hela tiden förnyas och på så sätt ”tvättar bort” oönskade gäster. Även om alla människor har detta skydd finns det stora skillnader mellan individer på hur infektionskänsliga de är.



Magsårsbakterien *Helicobacter pylori*

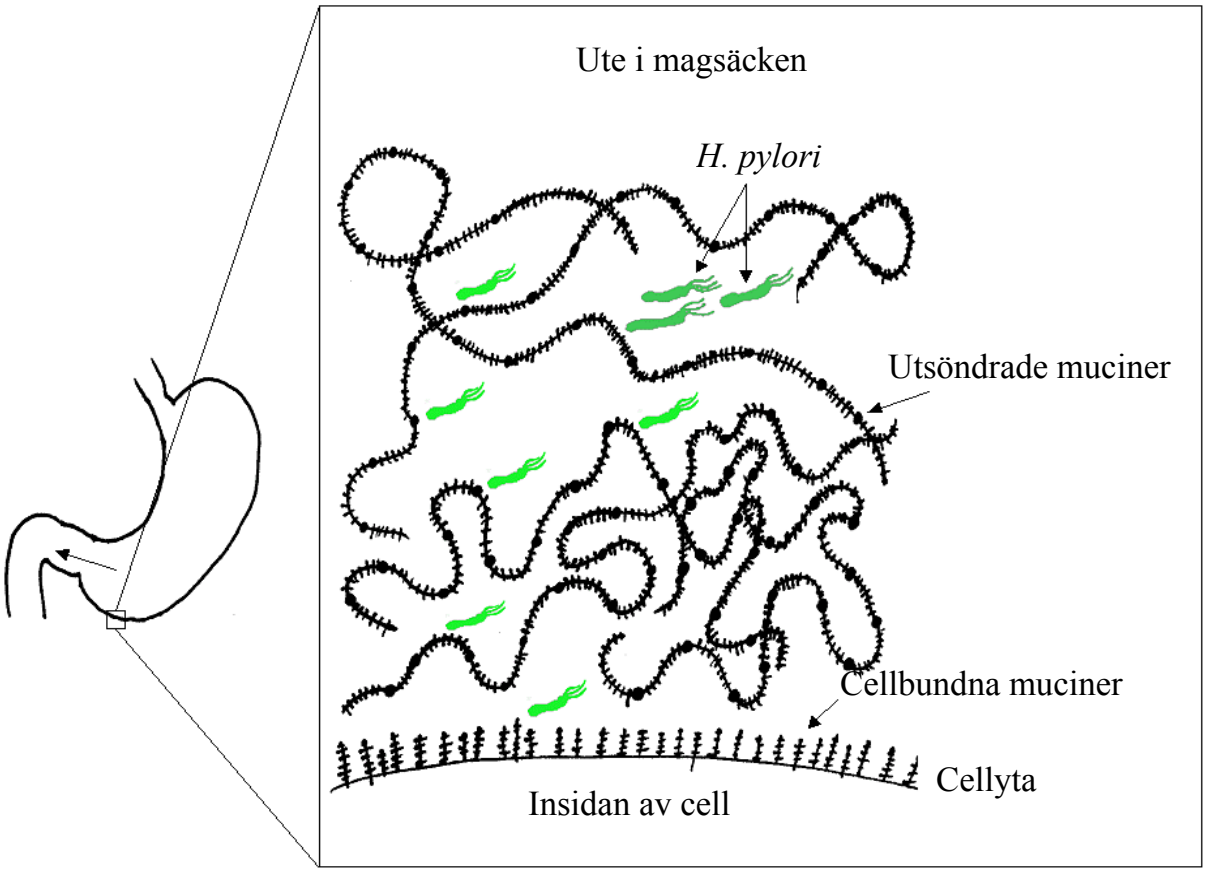
H. pylori (Bild t.v. tryckt med tillstånd från Ärztliche Praxis) orsakar en av världens vanligaste bakterie-infektioner och omkring 50% av världens befolkning beräknas vara infekterad. Bakterien orsakar kronisk inflammation i magslemhinnan, som kan övergå i magsår och cancer, och en *H. pylori*-infekterad individ beräknas ha ca 6 gånger så stor risk att utveckla magcancer som oinfekterade individer. 60-80% av magcancerfallen beräknas kunna förhindras, men man vet ännu inte vilka *H. pylori*-infekterade patienter man ska behandla eftersom endast 15% av infekterade individer visar symptom och 3% beräknas utveckla cancer. Ett problem i behandlingen av *H. pylori* är att den lätt blir resistent mot antibiotika, vilket gör att bakterien ofta återkommer efter behandling. Eftersom magen har som funktion att bryta ned födan (med hjälp av syra och nedbrytande enzymer) är den en relativt ogästvänlig miljö, och *Helicobacter* är hittills den enda kända bakterie som kan kolonisera en frisk mage. Till sin hjälp har de svansliknande rörelseorgan som de kan simma med och ett enzym som lokalt och tillfälligt kan neutralisera magsyran. Dessutom har *H. pylori* ett flertal strukturer på sin yta, så kallade adhesiner, som den använder för att binda till värdstrukturer. Med hjälp av adhesinerna kan bakterien ”låsa sig fast” i specifika strukturer på samma sätt som en rymdfärja dockar till en rymdstation. De mest välkarakteriserade adhesinerna är BabA (Blood-group binding adhesin) och SabA (sialic acid binding adhesin) vilka binder till kolhydratstrukturerna Le^b respektive sialyl-Le^x/sialyl-Le^a. Olika stammar/isolat av *H. pylori* bär på olika adhesiner.

Utsidan av insidan - magen skyddas av slem
Magsäckens insida är egentligen kroppens utsida eftersom material i detta utrymme, det vill säga maten, inte kommer in i själva kroppen utan transporteras igenom den som i en kanal. Magens slemhinna är täckt av ett slemlager, uppbyggt av stora glykoproteiner (muciner), som skyddar den underliggande vävnaden från magsyra och nedbrytande enzymer. I magen byggs detta slemlager upp av mucinerna MUC5AC och MUC6. Mucinernas struktur kan liknas vid en flaskborste där proteinkedjan är skaftet och kolhydratkedjorna borsten. Längst ut på ”borsten” sitter kolhydrat-strukturer som Le^b, sialyl-Le^x och sialyl-Le^a. De slembildande mucinerna består av flera sådana enheter som sitter ihop i långa trådliknande strukturer. Ytan på de celler som ligger under detta skyddande slemlager är dessutom täckt av en tät skog av kolhydratkedjor. Som en del av denna skog finns också cellbundna muciner som kan liknas vid en flaskborste som sitter fast i cellytan med skaftet (Se bild nedan). De är högre än andra cellbundna strukturer och sticker upp som höga träd ur lågväxande buskage. De kan dessutom signallera till insidan av cellen och kan därför överföra information från utsidan till insidan av kroppen. Det vanligaste cellbundna mucinet i magen är MUC1. Vid infektion återfinns majoriteten av *H. pylori* i slemlagret närmast slemhinnans yttersta cellager - epitelcellagret. Hur bakterien interagerar med slemlagret och epitelcellerna samt hur den kroniska infektionen uppkommer är ofullständigt känt.

Mitt forskningsprojekt handlar om vilka molekyler *H. pylori* binder till, via vilka strukturer detta sker, samt hur *H. pylori* påverkar magslemhinnan efter infektion. Fokus ligger på mucinerna och sockerstrukturer på dessa.

Fungerar de utsöndrade mucinerna som lockbete?
H. pylori med BabA adhesinet binder till en kolhydratstruktur som kallas Le^b på det slembildande mucinet MUC5AC och till H-typ-1 sockerstrukturen på det membranbundna mucinet MUC1. Det finns mycket mer MUC5AC än MUC1 eftersom MUC5AC, tillsammans med MUC6, ligger som ett tjockt lager över slemhinnan (Bild nedan). Konkurrens mellan interaktioner till utsöndrade muciner i mukuslagret och membranbundna muciner med signaleringsmöjlighet påverkar sannolikt infektionsförloppet. Om *H. pylori* fastnar i slemlagret kan den oskadliggöras genom att transporteras ut ur magen tillsammans med resten av maginnehållet (maten).

***H. pyloris* bindning till muciner beror på hur surt det är i magen**
H. pyloris förmåga att binda till muciner visades vara beroende av pH, det vill säga hur surt det är. Vid neutralt pH, som det är närmast slemhinnans cellyta, band *H. pylori*-stammar med BabA adhesinet till MUC5AC och MUC1 som ovan nämnt. Vid surt pH, dvs längre ut från cellytan i slemlagret, försvann denna bindning. Teoretiskt skulle detta innebära att bakterierna då kunde släppa taget till mucinerna som kommit ut i magsäcken och är på väg ut ur magen, och simma tillbaks in i slemlagret med mer neutralt pH. I verkligheten är det inte så enkelt eftersom andra mekanismer troligen motverkar detta, till exempel tar andra bindningsmekanismer över vid surt pH.



Magens yta skyddas av ett slemlager uppbyggt av muciner. Ute i magsäcken är pH surt, men i mukuslagret blir det gradvis mindre surt, och vid cellytan är pH neutralt. Vid infektion återfinns majoriteten av *H. pylori* i slemlagret närmast epitelcellagret, och bakterien binder både till cellbundna och utsöndrade muciner.

Ny bindningsmekanism upptäcktes!

Vid surt pH visar alla undersökta stammar liknande bindningsegenskaper, oavsett vilka adhesiner de har (BabA, SabA eller adhesion associated liporotein eller inget av dessa), och binder både till den andel av slembildande MUC5AC som har laddade kolhydratstrukturer och till en stor hittills oidentifierad komponent med laddade kolhydratstrukturer och som skulle kunna vara ett ”nytt” mucin.

Kolhydratstrukturerna som *H. pylori* binder till i magen förändras efter infektion

För att undersöka hur *H. pylori*-infektion påverkar magslemhinnan valdes rhesusapan som modell eftersom *H. pylori* makroskopiskt påverkar apan på samma sätt som människan. Rhesusapans muciner, kolhydratstrukturer samt interaktioner med *H. pylori* fanns dessutom vara mycket lika människans. Vävnadsprover som regelbundet tagits, före och under tio månader efter *H. pylori* infektion undersöktes. Vilka slembildande muciner som återfanns i magen förändrades inte under denna tidsperiod, däremot ändrades kolhydratstrukturerna på mucinerna. Förändringarna inkluderade en temporär minskning av Le^b och en ökning av sialyl-Le^a och sialyl-Le^x. Detta påverkar bakteriens interaktion med magslemhinnan då bakterien kan binda samtliga av dessa tre sockerstrukturer (Le^b är den struktur BabA binder till och sialyl-Le^a och sialyl-Le^x

de strukturer SabA binder till). Dessa glykosyleringsförändringar speglades även av att bindning med bakterier som bär på dessa adhesiner förändrades: bindning av BabA-positiva *H. pylori* minskade och SabA-positiva ökade som förväntat. Dessa resultat visar att *H. pylori*-infektionen förändrar värden på ett sätt som påverkar bakteriens förmåga att binda till magens yta.

Magens försvar

Ute i magsäcken råder en sur miljö, i mukuslagret blir det gradvis mindre surt och vid cellytan är pH neutralt. *H. pylori* har visserligen ett enzym som tillfälligt kan neutralisera syran i bakteriens närmiljö, men de kan bara motstå syra i ungefär en halvtimme. Därför skulle mekanismer som hindrar bakterien från att simma in till cellytan där det är neutralt pH kunna fungera som ett skydd mot *H. pylori*. Om de skulle fastna i något stort ute i lumen av magsäcken skulle de antingen dö av syran eller transporteras ut i tarmen med maten. Muciner från *Helicobacter*-infekterade apor har mer laddade strukturer, och binder mer *H. pylori* än muciner från friska individer, och efter infektion ökar mängden laddade kolhydratstrukturer (sialyl-Le^a och sialyl-Le^x) på mucinerna. Detta skulle kunna innebära att magen i försvar mot *H. pylori* producerar stora laddade molekyler som binder till bakterien ute i lumen av magsäcken och hindrar *H. pylori* från att simma in till cellytan där pH är förenligt med överlevnad. Mucinerna skyddar troligen magytans celler från bakterierna genom att de fångas i gelen och kan transporteras ut ur magen.

Kan den här forskningen leda till nya behandlingsformer?

Vid neutralt pH, binder *H. pylori* stammar som bär på BabA adhesinet till humant MUC5AC via kolhydratsstrukturen Le^b, och stammar med BabA adhesinet visar olika specificitet för MUC5AC med olika laddning på sina kolhydratstrukturer. Därigenom visar både *H. pylori* och dess värd på skillnader mellan individer i interaktionen. Dessutom blev aporna som saknade Le^b strukturen sjukare än de apor som hade detta socker. Efterssom *H. pylori* har stor förmåga att utveckla antibiotikaresistens är det är viktigt att identifiera vilka *H. pylori* stammar och vilka människor som är i riskzonen för att utveckla magsår eller magcancer och behandla dessa individer men ej de individer med ”snälla” *H. pylori* och en *H. pylori*-okänslig individuell profil. Min forskning skulle i förlängningen kunna leda till att man hittar bättre kriterier för vilka som ska behandlas eller ej och den grundbiologiska informationen som jag presenterat skulle kunna ge förutsättning för att utveckla nya mediciner, både för behandling av *H. pylori* infektion och andra patogener som koloniserar slemhinnan.

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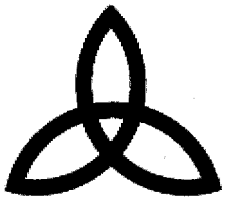

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